THE UPTAKE OF DISSOLVED OXYGEN BY FLOUR SUSPENSIONS

by

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SUMMARY.

The economic significance of wheat in human diet is largely attributable to the complex relationship which exists between its protein and lipid constituents. The technological properties of wheat flour are dependent on the susceptibility of these constituents to mild oxidation. The resulting reactions are of a complex nature but an evaluation of these reactions is a precursor to a more effective utilisation of this important crop as an article of diet.

The purpose of this study was to undertake an exploratory investigation into the uptake of dissolved oxygen by wheat flour suspensions. For this purpose flour suspensions were prepared by mixing a fixed quantity of flour and air equilibrated potassium chloride solution. An aliquot of the suspension was taken for study, and the uptake of dissolved oxygen was followed using a polarographic method. The electrodes were a rotating platinum microelectrode, which served as a cathode, and a saturated calomel electrode as anode. The potential applied to the cathode was regulated so that the current recorded on a sensitive ammeter was proportional to the concentration of oxygen in the suspension. Readings of oxygen concentration were taken at fixed intervals over the experimental period.

The results indicated that flour takes up oxygen rapidly when wetted. The amount taken up is influenced by commercial oxidative treatments and by the removal of flour lipids. The addition of extracted lipids and linoleic acid increased the uptake of defatted flour. A high level of an antioxidant (NDGA) did not inhibit the uptake mechanism. Sulphydryl blocking agents accelerated the uptake, but did not influence the overall amount of oxygen taken up by the flour. The addition of reduced glutathione increased the uptake of oxygen in the suspension. Impact milling did not affect the uptake, but differences were noted in the uptake of air classified high and low protein fractions. There was some evidence to suggest that the uptake process was pH sensitive. The results are discussed and related to the theories and findings of other workers.

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PREFACE

PREFACE

Wheat has a unique position among cereals, as it is only from wheat flour that satisfactory bread can be produced. When wheat flour is mixed with water to form a dough, it is found that the dough exhibits both elastic and extensible properties. These rheological properties are the principal factors in determining the suitability of wheat flour for bread making, as they confer on the dough the ability to be readily shaped and handled, and to retain the carbon dioxide produced during fermentation.

The plastic character of flour dough resides in the gluten complex, which consists of a three dimensional network of hydrated proteins and bound lipids. The physical properties of this complex may be influenced by the addition of small quantities of certain oxidising agents. The addition of oxidising agents to flour is a common practice in the milling industry to bring about optimum dough handling characteristics, and to produce a loaf of good volume and texture. The oxidising agents are believed to act either directly or indirectly on the gluten proteins of dough.

The chemical treatment of flour is a practice of long standing. As previously mentioned, certain oxidising agents have a beneficial effect on the character of dough and the finished loaf. These substances are termed improvers or improving agents. In addition to improvers, flour may be treated with chemicals which have a

bleaching action, this practice being due to a public preference for white bread. Only small concentrations of both improving and bleaching agents are required to bring about the desired effects and no adverse nutritional effects, in respect of their use, had been reported until 1946. At this time Sir Edward Mellanby (1) found that the incorporation of * 'Agenised' flour into the diet of dogs produced symptoms of canine hysteria. These investigations lead to an intensive reappraisal of the use of chemicals in flour treatment, and harmless alternative methods of bleaching and improving were sought.

In 1950, a patent was granted to Rank and Hay (2) for a process which enabled acceptable bread to be made from untreated flour. The Rank and Hay process involved the high speed mixing of a flour batter, prior to dough formation by the addition of a further quantity of flour. The bread obtained by this process was of good volume, texture and colour.

The chemistry of the Rank and Hay process was studied by Hawthorn and Todd (3). These workers found that oxygen was implicated in both the bleaching and improving effects. The bleaching effect was shown to be dependent on the enzymic oxidation of unsaturated lipids. The improving effect was believed to be due to a direct uptake of oxygen by flour protein.

The improving effect of oxygen during dough mixing had been reported previously in the literature (4-9); however its use in a commercial process stimulated research into this aspect of dough

* i.e. flour treated with nitrogen trichloride

oxidation. Two studies (10,11) based on the manometric measurement of oxygen, have indicated that lipid oxidation is the principal factor controlling the amount of oxygen absorbed during dough mixing. Recently, the rapid oxidation of both unsaturated and saturated fatty acids has been established in simple flour-water systems (12).

The work described in this thesis is concerned with the uptake of dissolved oxygen by flour suspensions. Essentially, this study has two aspects. These are concerned with the measurement of small amounts of dissolved oxygen in flour suspensions, and the inter-relationships of various flour components in the uptake process.

INTRODUCTION

INTRODUCTION

The subject of flour oxidation has occupied the attention of cereal chemists for many years, and in consequence a considerable volume of relevant literature has been published. The introduction has been divided into several sections, in order that the various approaches to the subject may be adequately presented.

Introduction of Oxidative Improving Agents in the Milling Industry

Flour is treated with oxidative improving agents in order to bring about optimum dough handling characteristics, and to produce a loaf of good volume and texture.

Probably the most widely employed improving agent is potassium bromate which was introduced in 1920 by Kohman, Irvin and Cross (13). It was found that the addition of a small amount of potassium bromate to a freshly milled flour produced a favourable result which could only otherwise be achieved by weeks of natural ageing. Potassium bromate has achieved popularity as an improver both in this country and the United States, and is generally incorporated into flour at a level of about 10-15 parts per million (14).

Hydrogen peroxide has been reported to improve the baking quality of flour (15), and Patterson (16) patented a process utilising this effect in 1921. The use of hydrogen peroxide has not achieved any popularity.

In 1921, Baker (17) introduced nitrogen trichloride or 'Agene', as a gaseous bleaching and improving agent. The use of this substance was widespread and continued until the investigations of Mellanby (1) in 1946. Mellanby found that 'Agenised' flour, when fed to dogs as a large percentage of the diet, induced symptoms of hysteria or running fits. The use of this substance was discontinued in Great Britain after 31st December 1955 following agreement between the Ministry of Food and the National Association of British and Irish Millers (18).

Chlorine dioxide has replaced nitrogen trichloride as a gaseous bleaching and improving agent. Its use was described by Staudt (19), who obtained a British patent covering treatment of flour with this oxidising agent. In 1933, an American patent (20) was taken out for the use of chlorine dioxide in combination with certain other oxidising agents. Ferrari et al.(21) reported that chlorine dioxide improved dough handling properties and loaf volume to a similar extent as nitrogen trichloride. Frazer et al. (22), after exhaustive investigation, came to the conclusion that chlorine dioxide produced no toxic substances in bread, and its use is now commonplace. The gas is usually generated by passing measured quantities of chlorine through a solution of sodium chlorite, and flour is treated at a level of about 15 parts per million (23).

Introduction of Bleaching Agents in the Milling Industry

Public demand for white bread was responsible for the introduction of bleaching agents at the turn of the century.

As early as 1879 a British patent (24) was issued for the bleaching of flour with chlorine. In 1899, Jago (25) found that ozone bleached the yellow flour milled from Oregon wheat. The treatment was not a commercial proposition, as the treated flour acquired a taint. Evenaso, both French (26) and British patents (27) were granted to Frichot for production of ozone by the flaming arc process to bleach flour. Avory (28) stated that the active agent was probably nitrogen peroxide and not ozone. In 1901, a patent was granted to John and Sidney Andrews (29) for the treatment of flour with nitrogen peroxide produced by the action of nitric acid on ferrous sulphate. When production of the gas by the flaming arc process was introduced for flour bleaching by Alsop (30), this method superseded the Andrews process for the generation of the gas. Nitrogen peroxide was the usual bleaching agent employed in mills until the introduction of nitrogen trichloride in 1921. Nitrogen trichloride or 'Agene' was in popular use for a period of thirty years until the investigations of Mellanby raised doubts as to the toxicity of 'Agenised' flour.

Chlorine dioxide, introduced in 1928, is not as effective a bleaching agent as nitrogen trichloride. In consequence, its bleaching action is usually supplemented by the addition of

about 30 parts of benzoyl peroxide per million parts of flour (23).

Theories of Improver Action

One of the earliest theories of improver action was advanced by Jørgensen (31-34), and Balls and Hale (35-37). These workers considered that flour contained latent proteolytic enzymes of the papain type, which could breakdown the gluten and destroy the gas retaining properties of dough. To exert their effect these enzymes had to be activated by a compound containing a sulphydryl group (-SH), such as glutathione or cysteine. These sulphydryl activators were only able to function in the reduced form; improvers were thought to oxidise the activators, thus suppressing proteolysis.

Sullivan, Howe and Schmalz (38) isolated glutathione (a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine) from a water extract of wheat germ. These authors suggested that glutathione may function as a proteinase activator, thus accounting for the deleterious effect of wheat germ on the baking quality of patent flour (39).

Jørgensen (40) supported his theory by experiments which indicated a decrease in the water soluble nitrogen of flour extracts treated with potassium bromate. These experiments could only be considered partially successful, as the amount of bromate necessary to produce this effect was in excess of that required for improvement.

Balls and Hale (41) concentrated the proteolytic enzyme from an extract of wheat bran and concluded that it was similar to papain. Hale (42) prepared the enzyme from patent flour.

The proteinase inhibition theory of improver action did not explain the findings of other workers in this field of research. Read and Haas (43) noted that bromate, in quantities applicable to commercial bakery practice, did not suppress the activity of wheat proteinase. Harris (44) found that wheat proteinase was not similar to papain in action.

Ford and Maiden (45) were of the opinion that glutathione had a direct softening action on gluten proteins. This view was supported by Sullivan et al. (46) who considered that the direct action on gluten was more important than the activation of a proteolytic enzyme. If the glutathione was oxidised by an improver this direct action on the gluten was not observed. These authors also proposed an alternative explanation of improver action. They considered that the oxidising improvers could bring about changes in the sulphur linkages of the gluten proteins with a consequent effect on dough characteristics. For several years conflicting evidence was published in support of both the proteinase theory and the theories based on direct oxidation of reducing substances and flour proteins. Ziegler (47-50) believed that the effect of various oxidising agents could be partially explained by the proteinase theory. Freilich and Frey (51-56) found that improver

action was more complex than a straightforward inhibition of proteinase activity. They showed that the 'excess bromate effect' (addition of bromate above a level of 60 p.p.m. which depresses loaf volume), could not be explained by the Jørgensen, Balls and Hale theory. According to the proteinase theory, the loaf volume should be progressively improved as the amount of bromate is increased, until complete inactivation of the proteinase has been effected. These workers concluded that oxidising agents act by:-

(i) Direct action on the gluten.

(ii) Oxidation of reducing substances.

(iii) Inhibition of proteolytic activity by action on enzyme or substrate.

During the period 1942-1943 several papers were published on various aspects of improver action. Evidence against the proteinase theory was advanced by Olcott, Sapirstein and Blish (57), and Sandstedt and Fortmann (58), whilst Smith and Geddes (59) suggested that potassium bromate acted directly on the gluten protein. In 1945, Howe and Glick (60) demonstrated the inhibition of wheat proteinase by sodium fluoride and hexyl resorcinol. Howe (61) found that these reagents had no effect on the rheological properties of dough. Howe and Glick (62) confirmed that the proteolytic activity of dough was not inhibited by potassium bromate in concentrations employed commercially. These findings indicated that the proteolytic theory improver action was not valid, and that a new theory was required which would take into account the direct effect of oxidising agents

on gluten.

Sullivan (63,64) proposed that the physical improvement brought about by oxidising agents could be explained by the formation of intermolecular disulphide bonds. These bonds were formed when sulphydryl groups on two adjacent protein molecules were oxidised. The more cross-links produced the greater the rigidity, toughness, and gas retention of the gluten. This view was consistent with the earlier experiments of Baker, Parker and Mize (65), who found that improvers lowered the sulphydryl content of flour water extracts and glutens.

Dempster et al. (66,67) considered that the improving effect of potassium bromate was due to two factors. The first of these involved oxidation of deleterious substances, such as glutathione, which would normally bring about undesirable changes in dough properties during development. The second factor was responsible for the positive improving action of bromate, and involved the activation of potential points of cross-linkage between the protein molecules. On shaping, or manipulation of the dough, the reactive centres were brought into close proximity and cross-links were formed.

The importance of the sulphydryl-disulphide system in determining dough properties was soon widely recognised. Glutathione, cysteine, sodium sulphite and bisulphite, all of which produce extreme extensibility and softness in dough, were considered to

function by scission of disulphide linkages (57,64,68,69). Matsumoto (70) measured the increase in sulphydryl groups brought about by treatment of a dough with bisulphite, using an amperometric titration.

In recent years, it has been reported that bromate does not account for complete oxidation of sulphydryl groups present in flour. Using radioactive tracers Lee, Tkachuk and Finlayson (71) reported that only 1 p.p.m. of bromate was reduced in a non-fermenting dough.

Matsumoto and Hlynka (72) found that bromate could bring about a small but definite decrease in the sulphydryl content of both water and acid soluble components of dough. The action of sodium sulphite increased the sulphydryl content of the water-soluble components, the effect on the acid soluble components (largely gluten proteins) being inappreciable. The results did not disprove the hypothesis that improvers act by oxidising the gluten proteins; however the water soluble proteins were also implicated in the oxidation. These workers pointed out that the oxidation of sulphydryl groups may be an adventitious reaction. Lee and Tkachuk (73) found that slurries of flour and freeze dried gluten reduced bromate to bromide, as did an aqueous extract of flour. The reduction brought about by the gluten slurry, and the aqueous extract, was less than that brought about by the whole flour slurry. The results supported the conclusion that bromate is a non-specific oxidising agent and may take part in reactions involving a number of flour constituents. Lee and Small (74)

studied the reduction of S^{35} labelled persulphate to sulphate in flour and dough systems. It was found that components other than gluten could undergo reaction with persulphate.

Strong evidence indicating that sulphydryl groups are involved in the bromate reaction was obtained by Bushuk and Hlynka (75). N-ethylmaleimide, iodoacetate and mercury all inhibited the bromate reaction due to their ability to combine with sulphydryl groups. It was not found possible to relate the sulphydryl content with the amount of reacted bromate.

In 1957, Goldstein (76) found that potassium bromate was ineffectual as a dough improver if the sulphydryl groups of the dough had been reacted with para-chloromercuribenzoate. Goldstein pointed out that because of the low sulphydryl content of gluten it was unlikely that two sulphydryl groups would be in close enough proximity to be oxidised to a disulphide bond. It was postulated that improvement may be explained by the action of the oxidising agent in hindering an exchange between sulphydryl and disulphide groups.

Frater, Hird, Moss and Yates (77) believed that the rheological properties of dough are directly related to the number of intermolecular disulphide bonds, and the rate at which they can exchange with sulphydryl groups. The findings of Goldstein were confirmed by Sullivan, Dahle and Nelson (78).

Axford and Elton (78a) considered that a substantial proportion of the work required to develop doughs by mechanical means was expended on the breaking of disulphide bonds. New disulphide bonds were thought to reform in such a way as to provide a stable, expandable, protein network, a process which would be facilitated by the presence of oxidising agents.

Bushuk and Hlynka (79) found that glutathione, cysteine, and sodium bisulphite, when incorporated into dough, increased the rate of bromate decomposition. The increase in reaction rate was proportional to the amount of the reagent added. The results were interpreted as support for the hypothesis that bromate reacts principally with sulphydryl groups, and that the number of sulphydryl groups was increased by the reagents studied. In a later paper, Bushuk and Hlynka (80) found that there was a faster rate of bromate decomposition in doughs from high protein flour compared with doughs from low protein flour. The results were thought to be related to the increased sulphydryl content of the high protein fraction, brought about by air-classification. Bushuk and Hlynka concluded that the sulphydryl groups of flour proteins are involved in the chemical improvement of flour quality. Whether this improving effect is produced by the cross-linking reaction as first proposed by Sullivan et al. (46), the modification of this reaction as proposed by Goldstein (76), or by some other mechanism, is still a matter for conjecture.

The Effect of Oxygen During Dough Mixing

(i) General Review of Early Observations

The introduction of oxidising agents by Kohman et al. (13) in 1920, to bring about the artificial maturation of flour, did not stimulate research into the effect of oxygen on flour or dough, as might be expected. It was not until 1937, that Baker and Mize (81) reported that doughs mixed in oxygen showed improvement, followed by deterioration if overtreated.

In 1939, Freilich and Frey (53) found that the depressing effect of cysteine, glutathione and wheat germ could be overcome by mixing in oxygen. These authors believed that these substances activated proteolytic enzymes, or exerted a direct detrimental effect due to their reducing character. On extending the study (54) it was reported that flours vary in their oxygen requirements. Some flours responded more readily to oxygen than bromate, suggesting that there were differences in their action in doughs. In 1947, the effect of oxygen on dough development and bread quality was studied (82). Mixing in oxygen was found to improve the rheological properties of doughs, and produced an increase in loaf volume. The beneficial effect of oxygen, which was noticeable during the first few minutes of dough mixing, was independent of proteinase inhibition. To determine if the oxygen effect resulted from enzyme activity, a dough containing 0.5% cuprous chloride was mixed in oxygen. The cuprous chloride was expected to act as an enzyme inhibitor, although it was a poor choice of

reagent as traces of copper may catalyse the oxidation of sulphydryl groups (128). The properties of the dough were similar to those of a control mixed in nitrogen, and this was presented as inhibition of the oxygen effect. Further evidence for an enzymic mechanism was obtained by adding small amounts of quinua flour (from Quinua polyepsis) to the dough. Quinua flour appeared to have a high concentration of the oxidising enzyme, as a dough containing 10% of this flour showed a typical oxygen effect when mixed in air. If the quinua flour was heated to 95°C, before being incorporated into the dough, the oxygen effect was not observed, indicating that the activity of the enzyme system had been denatured by heat.

The improving effect of oxygen and the mechanism whereby it is brought about, were matters of academic interest until 1946 when the investigation of Mellanby (1) stimulated a re-examination of the subject.

In 1950, Rank and Hay (2) patented the 'batter' or aeration process, which relied on high speed mixing to achieve both a bleaching and improving effect. By this process bread could be prepared of equal quality to that obtained from conventionally treated flour. The bleaching effect was attributed to the lipoxidase activity of the flour, whilst the improvement was considered to be due to mechanical work performed on the batter.

In order to appreciate the complex effect of oxygen during the mixing of flour doughs, the literature concerning the bleaching and improving mechanisms are considered separately.

(ii) The Bleaching Effect of Oxygen during the mixing of Flour Doughs and Batters

In 1934 Haas and Bohn (83) developed a commercial product known as 'Wytase' based on unprocessed soya flour. It was claimed that when this product was incorporated into a dough, a bleaching effect was observed. Bohn and Favor (84) attributed the bleaching effect to the unsaturated fat oxidase activity of the soya, but did not offer any direct evidence to support this view.

The presence of an enzyme which could catalyse the oxidation of unsaturated lipids had been demonstrated by André and Hou (85), and Craig (86) in soya beans and other plants. Sumner and Sumner (87) demonstrated that this enzyme, lipoxidase, was responsible for the simultaneous bleaching of carotenoids during the oxidation of unsaturated lipids. Later work has shown that lipoxidase is a highly specific catalyst for the oxidation of unsaturated fatty acids containing cis-methylene-interrupted double bonds, such as linoleic and linolenic acids (88,89). Carotenoids are bleached due to abstraction of hydrogen by free radicals, which are intermediates in the formation of the fatty acid hydroperoxides (90).

The presence of lipoxidase in wheat germ was detected by Sumner in 1943, (91), but the quantity present was only 2.3% of that found in soya bean meal. The lipoxidase activity of wheat flour was demonstrated by Miller and Kummerow (92), using a carotene destruction method. Irvine and Winkler (93) showed that lipoxidase was responsible for the destruction of the yellow

pigments of durum semolina during macaroni processing.

When Rank and Hay (2) observed that high speed mixing produced a bleaching effect, which could be further enhanced by the addition of 0.1% of unprocessed soya, the implication of lipoxidase in the bleaching action was suspected. Strong evidence for a lipoxidase catalysed bleaching effect was obtained by Hawthorn and Todd (3), who studied the chemistry of the aeration process. These workers demonstrated:-

(i) Addition of lipoxidase (in the form of unprocessed soya flour) gave rise to increased bleaching.

(ii) Addition of linoleic acid increased the degree of bleaching.

(iii) The degree of bleach, in the absence of an added lipoxidase source, increased with increasing time of high speed mixing.

Further, it was shown that addition of a haematin compound (eg.catalase) had a similar effect to lipoxidase. This finding was in agreement with the observation of Tappel (94), and Banks (95), who have shown that haematins may act as unsaturated fat oxidases in the presence of traces of preformed peroxide. The role of high speed mixing in bleaching was considered to provide oxygen for the action of unsaturated fat oxidases, especially lipoxidase.

(iii) The Improving Effect of Oxygen during the mixing of Flour Doughs and Batters

Baker and Mize (81) reported that doughs mixed in an oxygen atmosphere showed a definite improvement effect. Freilich and Frey (53,54,82) found that an improvement in loaf volume and texture could be achieved by mixing doughs in oxygen. The effect became apparent during the first few minutes of mixing, and it was suggested that an enzymic reaction was responsible, but little convincing evidence was offered for this view. Smith and Andrews (96) obtained rheological data which indicated that oxygen had an immediate effect, and a time dependent effect, in dough. Dempster, Hlynka and Anderson (97) confirmed this two-stage reaction, and found that the magnitude of both stages was related to the oxygen concentration in which the doughs were mixed.

The Rank and Hay process brings about an improvement in bread quality besides having a bleaching effect. Hawthorn and Todd (3) considered that there were two possible explanations for the improvement observed in the aeration process. These were:-

(i) Work strengthening of the gluten by the expenditure of mechanical energy on the dough, as proposed by Rank & Hay (2).

(ii) Chemical reactions made possible by the incorporation of air into dough during high speed mixing.

It was found that mixing per se was not responsible for the improvement, as the loaves produced by mixing under a nitrogen atmosphere were smaller than those produced if mixing was carried out in air. Further, it was demonstrated that conventional mixing in an oxygen atmosphere brought about similar or even greater effects than high speed mixing in air (98).

The effect of oxygen on doughs indicates that oxygen is involved in chemical reaction with flour constituents. The task of discovering which substances have the role of oxygen acceptors in doughs is made difficult by the complex nature of flour. It has already been mentioned that the bleaching effect in the aeration process is due to the coupled oxidation of linoleic and linolenic acid under the influence of lipoxidase; consequently these fatty acids are oxygen acceptors in doughs. The improvement effect in the aeration process has been suggested to be due to direct uptake of oxygen by flour protein, thus indicating a further centre for oxidation. The role of flour lipids and proteins in oxidative improvement will be discussed in the following section, in order to provide a general background for recent work on the oxygen effect.

The Role of Flour Lipids in Oxidative Improvement

The early literature contains several references to the baking behaviour of bread made from flour extracted with fat solvents. The results were of a conflicting nature, some authors (99-105)

reporting improvement in baking quality, whilst others reported a deterioration (106 - 109). In 1956, Cookson and Coppock (110) found that volume could be slightly improved by using defatted flours. The improvement has been shown to be negligible in recent years, probably due to a change in the flour extraction rate and varying climatic conditions (111).

In 1940, Sullivan, Howe, Schmalz and Astleford (46) observed that treatment of flour with oxidising agents reduced the amount of lipid capable of being extracted from doughs. Cookson and Coppock (110) demonstrated that flour, which had been extracted with carbon tetrachloride, showed similar characteristics to flour which had been treated with an oxidative improver. Defatted flour doughs required less bromate than control flour doughs to bring about the same degree of improvement. The authors pointed out that carbon tetrachloride did not extract the entire lipid, and the flour might be substantially inert to oxidising improvers in the complete absence of lipids. The findings of Sullivan et al. (46) were confirmed in that the recovery of lipid from treated flours was smaller than for untreated flours, also changes were observed in the ultra-violet absorption spectrum of lipids from chlorine dioxide treated flours. It was concluded that any comprehensive theory of oxidative improvement should take into account the lipids, as well as the proteins of flour. In a later paper, Cookson et al. (111) showed that replacement of extracted lipids to defatted flour did not restore the

more extensible nature of the unextracted flour. It was considered that the extraction procedure had altered the properties of the lipids, or the nature of the lipid-protein linkage. The effect of chlorine dioxide treatment was less marked in dough characteristics (though not in loaf volume) of a defatted flour, compared with an undefatted flour.

The relationship between flour lipids and the bromate reaction in dough was studied by Cunningham and Hlynka (112) with very interesting results. These workers found that extraction of flour with fat solvents decreased the rate of bromate decomposition in doughs. The addition of the lipid extract restored the rate to its original level, providing the flour had not been damaged by the solvents. Lipids were thought to function as intermediates between bromate and reducing substances, the nature of the mediation residing partly in the chemical transport of bromate oxidising power, eg. peroxide formation, and partly in the structural role of lipids in flour. Traub, Hutchinson and Daniels (113) have shown, from X-ray studies, that the protein fibres of wheat flour are held together by layers of phospholipid. Since bromate action has been traditionally associated with protein, such an arrangement could provide a basis for lipid mediation in the bromate reaction.

Lee and Tkachuk (114) confirmed that defatting with petroleum ether reduced the amount of bromate (Br^{82}) converted to bromide. The reaction of bromate with lipids was held to supplement any

primary reaction, such as the formation of disulphide linkages, which would consume only a small fraction of the total bromate added to the flour. In a subsequent paper (73), these authors found that bromate was not reduced to bromide by a dioxane solution of petroleum ether soluble flour lipids, indicating that there is no direct reaction between bromate and lipids in dough.

Recently, Bushuk and Hlynka (80) have related the reduction of bromate to the presence of oxygen during dough mixing. A control dough, and a petroleum ether defatted dough, mixed in air showed a much lower conversion of bromate to bromide than the corresponding doughs mixed in nitrogen. On comparing the extracted and control doughs mixed in nitrogen little difference was observed in the degree of bromate reduction. This was not the case with the air mixed doughs, as the extracted flour doughs showed a smaller reduction of bromate compared with the control. These results were explained on the basis that crude lipid and sulphydryl groups compete for oxygen. The removal of the available oxygen, by the lipoxidase catalysed mechanism (115), allows the bromate reaction to proceed uninhibited. The lipid hydroperoxides, which were formed as a result of lipid oxidation, were considered to compete with bromate for sulphydryl groups present in the flour. Antioxidants were found to depress the bromate reaction, and it was thought that the oxidation product reacted with sulphydryl groups, as was the case with lipids.

The competition between lipid oxidation products and bromate for the sulphydryl groups of dough will be discussed in a later section.

The Role of Sulphydryl Groups in Oxidative Improvement

Sullivan, Howe, Schmalz and Astleford (46) were the first to draw attention to the possible role of sulphydryl groups in oxidative improvement. Baker, Parker and Mize (65) reported that nitrogen trichloride and chlorine treatment lowered the sulphydryl content of both a water extract of flour and a gluten dispersion. Mixing in oxygen progressively lowered the sulphydryl content of a water extract of dough over a period of 24 minutes, after which the value became constant. Mixing in a carbon dioxide atmosphere had no effect on the sulphydryl content of either material.

Sullivan (64), considered that improvement was brought about by oxidation of adjacent pairs of sulphydryl groups to disulphide bridges, thus producing cross-linkages within the protein structure. Goldstein (76) considered it improbable that pairs of sulphydryl groups could be so aligned that oxidation could easily occur, and postulated an exchange mechanism between sulphydryl and disulphide groups. By this mechanism, free sulphydryl groups are brought into the proximity of disulphide bonds during mixing. An interchange of sulphydryl groups occurs, and thus new disulphide bonds and sulphydryl groups are formed. The original disulphide bond was considered to

be opposed to mixing shearing forces, and the new bond is so orientated as to longer oppose these forces, consequently a relaxation occurs within the dough. The improvement of dough by oxidising agents is due to a hindrance of the exchange mechanism, as the improver removes sulphydryl groups from the system, thus preventing relaxation occuring. To test this hypothesis, Goldstein blocked the sulphydryl groups of a dough with para-chloromercuribenzoate, and demonstrated improvement in the mechanical properties of the dough. The addition of improvers to a dough with all the accessible sulphydryl groups blocked was without effect.

Mecham (116) clearly demonstrated that sulphydryl groups play a governing role in dough mixing behaviour. Doughs containing specific sulphydryl blocking agents (N-ethylmaleimide, para-chloromercuribenzoate, iodoacetamide) showed similar rheological properties to those treated with oxidising agents.

Matsumoto and Hlynka (72) found that the water soluble protein components of flour contained two to three times as many sulphydryl groups as the fraction soluble in 0.01N acetic acid (largely gluten proteins). Bromate and iodate decreased the sulphydryl content of both fractions, although mixing in air only slightly decreased the sulphydryl content of the water soluble fraction. Sulphydryl blocking agents decreased the sulphydryl content of both fractions, para-chloromercuribenzoate being slightly more effective than

N-ethylmaleimide. The results obtained with blocking agents revealed the presence of inaccessible sulphydryl groups which had not reacted. The authors pointed out that the water soluble proteins, as well as the gluten proteins of flour, should be considered in a comprehensive theory of oxidative improvement. Mecham, Sokol and Pence (117) demonstrated that it was the gluten fraction which determined the effect of N-ethylmaleimide on dough mixing properties, rather than the soluble fraction.

Sokol, Mecham and Pence (118) found the sulphydryl content of bromated doughs, mixed in air, was similar to that of untreated doughs, except in the case of a fifth break flour, when a small decrease was observed on the addition of bromate. The authors noted that the reactive sulphydryl groups may have been oxidised by atmospheric oxygen before measurements of the effect of bromate could be recorded. The same authors (119) reported that there was a rapid loss of sulphydryl groups during the first few minutes of dough mixing. For a 20 minute period, sulphydryl losses varied from 38% to 64% of the original sulphydryl content, depending on the flour studied. In most cases the sulphydryl content had reached a constant value after 20 minutes, most of the decrease occurring during the initial period of mixing (0-5 minutes). From a study of dough mixing stability it was found that a correlation existed between rate of sulphydryl loss and mixing stability. The more stable doughs lost sulphydryl groups faster than the less stable doughs. Flour suspensions

had a lower rate of sulphydryl loss than doughs, from which it appeared that sulphydryl groups failed to become available for reaction in suspension.

Recently, Sullivan, Dahle and Larson (120) reported that the sulphydryl groups of wheat flour appeared to be equally distributed between the water soluble proteins and gluten proteins. This is in contrast with the results of Matsumoto and Hlynka (72). No detectable sulphydryl groups were found in a water-saturated butanol extract, although the extraction had been carried out under nitrogen.

Hird and Yates (121) have demonstrated that buffered solutions (pH 6) of glutathione and cysteine were oxidised to the extent of 1.6% and 13% respectively by air, and to a slightly greater extent by bromate at a level used in flour treatment.

The accessible sulphydryl groups of doughs have been considered to be the groupings oxidised by improving agents (119,122). Measurement of these groups was carried out by Bushuk (123) using an iodate titration method. The results indicated that the accessible sulphydryl content increased with increasing protein content of flour, the values ranging from 5 - 10 µeq./g. protein. In dilute flour slurries (i.e. 50 parts of water to 1 part of flour) a limiting value of 10.4 µeq./g. protein was recorded. This value is slightly higher than the value of 8.1 µeq./g. protein observed by Sokol, Mecham and Pence (124), for the total

sulphydryl content of flour dispersions.

Tsen and Bushuk (125) have found that 30 p.p.m. of potassium bromate did not produce any significant effect on the sulphydryl loss of doughs mixed in nitrogen. This low rate of reaction was in agreement with earlier findings (119,121) and the authors considered that the bromate reaction in dough is very slow. Potassium iodate had a much faster reaction with sulphydryl groups, and oxygen competitively inhibited the reaction at low iodate concentrations. The disulphide content of the dough was fouhd to be about 15 μeq./g.flour or 100 μeq./g. protein, and this did not vary during mixing in oxygen and air. It was found that iodate treated doughs mixed in oxygen for 15 min. did show a disulphide loss. The authors suggested that this loss may be responsible for the breakdown process produced by the prolonged mixing of over-oxidised doughs.

Sullivan, Dahle and Schipke (126) have recently summarised the current theory of oxidative improvement as follows:-

> 'Improvers and specific sulphydryl reagents inhibit the exchange reaction between RSH and RS-SR by oxidising or blocking some thiol groups which otherwise would cause too great extensibility. In addition, oxidising agents may react on RSH to form new disulphide bonds, thus strengthening the dough. Reducing agents split the interchain S-S bonds causing softness and extensibility'.

From observations of the labile and non-labile sulphydryl groups in flour and dough, these authors put forward an additional hypothesis on the improvement process. It was shown that about 50% of the total sulphydryl content of flour underwent rapid oxidation during dough mixing. These sulphydryl groups were considered to be those of water- and salt-soluble proteins, and the remaining sulphydryl groups were not reactive under conditions of mixing. It was postulated that the labile sulphydryl groups of non-gluten proteins were involved in an exchange reaction with gluten disulphide groups, which weakened the dough matrix. Removal of the labile sulphydryl groups by oxidation prevented the exchange reaction occurring, thus increasing the strength of the dough. As pointed out by these workers it has not been possible to prove unequivocally any theory of oxidative improvement, and more information is required on protein structure before a clearer picture will emerge.

The Uptake of Oxygen by Flour Doughs and Batters

The early references to the improving effect of oxygen, during dough mixing, have already been discussed (53,54,81,82,96,97). These authors were studying dough rheology, and no definite observations were made concerning the biochemical systems involved in the oxidation phenomenon. Freilich and Frey (82) did present some rather dubious evidence which suggested that the oxygen effect was enzymic, but it is only recently that attempts have been made to elucidate the mechanism of the uptake process.

In 1956, Bungenberg de Jong (127) found that gluten, though extracted with petroleum ether, became rancid when exposed to oxygen and light. The rancid odour was considered to be due to the oxidation of unextracted lipids, which were tightly bound to the gluten surface. In further experiments, the rheological properties of extracted flour doughs were studied. The doughs appeared to be very sensitive to atmospheric oxidation, the oxidation being enhanced by high oxygen tensions or traces of copper ions. The author was of the opinion that direct oxidation of gluten was hampered by the presence of unsaturated lipids. If the flour had been extracted by an efficient solvent, the gluten would undergo rapid oxidation. It is not clear from the published data which solvent had been used for the extraction of the flour, consequently the effect of residual lipid oxidation cannot be neglected. Further, the improvement effect of copper ions in unextracted doughs is

well known, and is probably related to the oxidation of sulphydryl groups or the formation of stable mercaptides (124,128).

In 1955, Hawthorn and Todd (3) concluded that the improvement effect, observed in the Rank and Hay process, was due to direct uptake of oxygen by flour protein, and was independent of unsaturated fat oxidase activity. This conclusion was based on the observation that improvement still occurred, even though the flour had been defatted with petroleum ether. As pointed out by Glass (129), and Learmonth (130), petroleum ether would not have completely removed all the lipid from the flour. The latter author considered that gluten oxidation was dependent on the prior oxidation of unsaturated lipids by a lipoxidase or haematin catalysed system. Mapson and Moustafa (131) demonstrated that the lipoxidase of ungerminated peas could bring about the coupled oxidation of glutathione in the presence of unsaturated lipids, and such a reaction may play a role in improvement during high speed mixing.

The first attempt to measure the oxygen uptake of a flour suspension was made by Cosgrove (10) in 1956. The apparatus consisted essentially of a manometer connected to a flask in which flour (25 g.), and a phosphate buffer (pH 6.5, 40 ml.), were mixed under nitrogen. The entire apparatus was immersed in a thermostatically controlled water bath at 25°C. The flour suspension was stirred for 5 minutes, then the nitrogen was replaced by air, and readings were taken on the manometer every 5 to 10 minutes over

a period of 1 hour. It was found that the flour batter absorbed oxygen rapidly for about 20 minutes, thereafter the uptake gradually declined. The amount of oxygen taken up by suspensions was of the order of 600-1000µl. O_2 after 20 minutes from the commencement of the experiment. Extraction of the flour with light petroleum ether markedly reduced the ability of the batter to take up oxygen, although a residual uptake was still recorded. If the extracted lipid was replaced the oxygen uptake was restored, and lipid from stored flour was more effective than lipid from freshly milled flour in this respect. Heat treatment of defatted flour destroyed the uptake mechanism, as addition of the extracted lipid did not restore the uptake. Cosgrove was of the opinion that oxygen absorption was due to the lipoxidase activity of flour, which had been studied earlier by Irvine and Anderson (132). Cosgrove pointed out that extraction with light petroleum ether did not remove all the lipids from flour, and the residual uptake noted with defatted flours may be responsible for the improvement in the Rank and Hay process (2) observed by Hawthorn and Todd (3).

In 1957, Smith and Andrews (11) investigated the uptake of oxygen during the mixing of flour doughs. A manometric method was employed, the manometer being attached to a 50 g. farinograph mixing bowl fitted with a gas tight cover. Water and flour were mixed under an atmosphere of oxygen, and readings were taken from the commencement of mixing. The graphs of oxygen uptake

against mixing time indicated that oxygen absorption occurred rapidly and reached a maximum value after about 20 - 25 minutes of mixing. The amount of oxygen absorbed by a patent flour dough was of the order of 8000 µl. O_2 after 20 minutes from the commencement of mixing. Lower grades of flour took up substantially more oxygen, for example a first clear flour absorbed twice as much oxygen over the same period as the patent flour. Extraction of the flour with pentane (b.p.36° - 40°) reduced the oxygen uptake; however, readdition of the extracted lipid restored the uptake to its original value. There was no direct correlation between the fat content of the flour and the amount of oxygen absorbed, but a linear relationship was established between free fatty acid content and oxygen uptake.

Smith and Andrews (11) carried out further experiments which indicated that the agent catalysing the oxygen uptake was water soluble and heat senstive. The optimum pH for oxygen absorption was between 6 and 7, which is in the range reported optimal for lipoxidase activity by Irvine and Anderson (132). The oxygen uptake of flour steadily increased during storage at room temperature, while the uptake of flour held at -20°F. remained constant. Analytical evidence showed that there was a direct connection between oxygen uptake and the development of free fatty acids in the flour.

In a second paper (133), the study was extended to relate the effects of oxygen and lipids to the physical and chemical properties of flour doughs. It was found that the effects of sulphur dioxide and sodium bisulphite, which decrease the mixing tolerance of doughs, were enhanced by defatting the flour. If the lipid was returned to the treated flour the mixing tolerance was restored, although it was necessary for the mixing to be carried out in the presence of air. On adding various lipid fractions to defatted flour doughs, it was shown that the free fatty acid fraction was responsible for counteracting the weakening effect of bisulphite. Linoleic and linolenic acids were able to buffer the effect of bisulphite, whereas oleic and stearic acids produced no response. Oxygen was required to observe the buffering effect of linoleic acid, however, the addition of oxidised linoleic acid to a nitrogen mixed dough produced a similar result. The oxidation product of the poly-unsaturated fatty acids was considered to be responsible for the buffering effect of flour lipids.

The authors state that the oxidised poly-unsaturated fatty acids may act directly against the reducing agent in the dough, or alternatively, have an oxidising effect on a dough component. It is known that sulphite and bisulphite produce a softening effect through scission of disulphide groups (134), which would provide potential substrates for lipoxidase-coupled oxidations.

Smith, van Buren and Andrews (133) also compared the properties of extracted and unextracted flour doughs using the extensograph which measures the resistance to extension of a dough and its extensibility. It was found that extracted first and second clear flour doughs showed a greater response to mixing in oxygen than the corresponding unextracted controls. The oxygen uptake of the extracted doughs was much smaller than the controls, indicating that dough properties could be influenced by small amounts of oxygen in the absence of free lipids. The mixing characteristics of the control doughs could be restored by the addition of linoleic acid to the extracted flour. Oleic acid did not restore the mixing pattern, therefore poly-unsaturated fatty acids were apparently responsible for the different behaviour of the two doughs.

Hawthorn and Todd (3) had shown that defatted flour doughs responded to molecular oxygen, an effect which was considered to be due to direct uptake of oxygen by flour protein, and independent unsaturated fat oxidase activity. The results of Smith, van Buren and Andrews (133) also show an oxygen mixing response with defatted flour doughs. The poly-unsaturated fatty acid content of the doughs was related to the amount of oxygen taken up during mixing, and the unsaturated fat oxidase system had an influence on the mixing characteristics of the dough.

Mixing doughs under oxygen increased the loss of sulphydryl groups compared with controls mixed under nitrogen. The rate of loss of sulphydryl groups, in a second clear flour dough, was decreased after lipid extraction. This finding was related by Smith et al. (133) to the work of Mapson and Moustafa (131), who demonstrated the oxidation of glutathione in the presence of the lipoxidase-fatty acid system from ungerminated peas. A sulphydryl loss was detected in nitrogen mixed doughs, which was considered to be due to minute traces of residual oxygen acting on labile sulphydryl groups.

To summarise it may be stated that both Cosgrove (10), and Smith and Andrews (11), ascribe the oxygen uptake in suspensions and doughs to lipoxidase activity. Further, Smith et al. (133) consider that the fatty acid oxidation product may be involved in the oxidation of sulphydryl groups during dough mixing.

In a re-appraisal of the oxygen effect, Hawthorn (135) has pointed out that the apparatus of both Cosgrove and Smith et al. was insensitive to small changes in oxygen concentration. Hawthorn was of the opinion that no conclusive evidence had been advanced relating unsaturated fat oxidase activity with the uptake of small amounts of oxygen concerned in improvement by the Rank and Hay process.

Morrison (12) has recently criticised the findings of Smith and Andrews (11). This worker pointed out that the lipoxidase

catalysed oxidation of linoleic and linolenic acids could only account for an oxygen absorption of 2100 µl. O_2/50 g. flour, based on a normal free fatty acid content. Smith and Andrews (11) reported oxygen absorption figures of about 2-4 times this value, which indicated the oxidation of other flour constituents besides linoleic and linolenic acids. Morrison (12), using a gas chromatographic technique, was able to demonstrate the general loss of free fatty acids during the mixing of flour-water sponges. It was suggested that the free fatty acid losses were due to lipoxidase catalysed oxidation of essential fatty acids (linoleic + linolenic acids), and concurrent enzymic oxidation of all free fatty acids. The addition of a high level of nordihydroguaiaretic acid, an antioxidant, to the flour-water system did not inhibit the lipoxidase oxidation, and in fact increased the loss of essential fatty acids. This may have been due to an acceleration in the decomposition of linoleate hydroperoxide brought about by the high level of antioxidant (136).

Thus, the uptake of oxygen by flour doughs and sponges has been attributed to lipoxidase activity (10,11), lipoxidase activity and concurrent free fatty acid oxidation (12), and lipoxidase activity and direct uptake by flour proteins (3). The relative magnitude of these systems, and their significance in dough improvement, remains to be established.

Recent Work on the Inter-relationship of Lipid and Sulphydryl Oxidations

Recent research has indicated that inter-relationships may exist between dough oxidation systems. A review of this work aids in unifying the various sections of this introduction, and represents the state of knowledge at the present time.

Cunningham and Hlynka (112) found that the presence of air or oxygen inhibited the rate of bromate decomposition in dough. On extracting the flour with petroleum ether the role of oxygen as an inhibitor was accentuated. The competitive inhibition of the bromate reaction by oxygen was confirmed by Bushuk and Hlynka (75). In a later paper (80) these authors postulated that lipid competed with protein sulphydryl groups for oxygen, and that defatting enhanced the inhibitory effects of oxygen on the bromate reaction. Lipid hydroperoxides, which are formed as a result of lipoxidase activity, also competitively inhibited the bromate reaction by oxidising sulphydryl groups. The postulates of the authors may be set out as follows:-

(i) Bromate + Sulphydryl Groups ⟶ Oxidation Products
e.g. S-S bonds formed.
Main reaction of bromate in dough, and presumably responsible for improvement.

(ii) Oxygen + Sulphydryl Groups ⟶ Oxidation Products.
Oxygen competitively inhibits bromate decomposition.

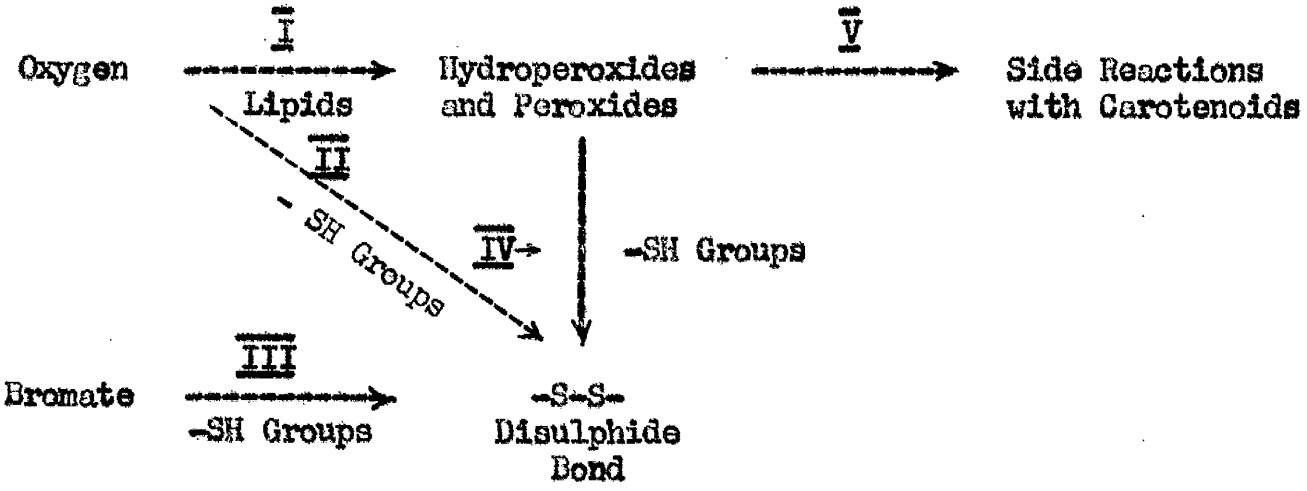
(iii) Oxygen + Lipids ⟶ Hydroperoxides (via lipoxidase catalysed mechanism). Presence of lipids decreases inhibitory effect of oxygen on the bromate reaction.

(iv) Hydroperoxides + Sulphydryl Groups ⟶ Products. Hydroperoxides competitively inhibit bromate decompositio

Tsen and Hlynka (137) measured lipid peroxidation during dough mixing by a modified thiobarbituric acid method. Definite evidence was obtained that 'free' lipid (i.e. that extractable with petroleum ether) was oxidised during the mixing process. The amount of peroxide formed could be increased by the addition of a lipoxidase preparation, in agreement with the earlier results of Smith and Andrews (11). Nordihydroguaiaretic acid inhibited the formation of peroxides in the dough after five minutes mixing in oxygen. The addition of peroxides and hydroperoxides to the dough produced a strengthening effect as measured by rheological properties. These authors considered that peroxides and hydroperoxides strengthen dough by oxidising sulphydryl groups, as it is known that sulphydryl groups are involved in determining the rheological properties of dough (75,77,116). The presence of sulphydryl blocking agents, or improvers, increased the

degree of lipid peroxidation. This was put forward as evidence that sulphydryl groups and lipids could compete for the available oxygen in the dough. If the sulphydryl groups were oxidised, or blocked, then more oxygen would be available for lipid oxidation.

The relationship between oxygen, lipids and sulphydryl groups, according to Tsen and Hlynka, may be expressed in the form of the following scheme:-



Reactions I & II — competitive for oxygen

Reactions II & III — competitive for sulphydryl groups

Reaction IV — dough strengthening effect observed on the addition of peroxides to dough.

Reaction V — proposed by Narayanan and Hlynka (138) to account for low effect of lipid peroxides as improvers.

Narayanan and Hlynka (138) found that mixing in oxygen brought about a marked improvement effect in doughs from defatted flours. This greater apparent effect of oxygen on defatted flours has also been noted by other authors (110,133), and Narayanan and Hlynka were of the opinion that lipids exert a protective action against the improver effect of oxygen. If the lipids were removed, more oxygen was available to react with flour sulphydryl groups. The addition of a petroleum ether extract of flour lipids restored the protective action against the improver effect. Oleic and linoleic acids brought about an initial improvement effect followed by the protective action. The reason for the improvement was unknown, but the protective action was considered to parallel the formation of peroxides in the dough. Antioxidants, when added to normal and defatted flours mixed in air, produced a slight improvement effect, which was believed to be due to the oxidised antioxidant acting as an improver and oxidising sulphydryl groups. The authors found that the addition of bromate to defatted flours brought about a further improvement on mixing in air. This finding is in contrast to previous results (75,112), that oxygen had an inhibitory effect on the reaction rate of sulphydryl groups with bromate.

Recently Tsen and Hlynka (139) demonstrated that doughs prepared from defatted flours had a faster rate of sulphydryl loss than doughs from the original flour. The doughs had been mixed

for 2.5 min. in air or oxygen, then allowed to rest. If the doughs were mixed continuously in air or oxygen for 20 min. it was found that the original flour dough had the more rapid sulphydryl loss. The results were explained on the basis of the scheme outlined earlier (137). Direct competition for oxygen occurred between lipids and sulphydryl groups in the resting doughs. Removal of lipids would, therefore, bring about a greater sulphydryl loss. In the case of doughs mixed for a longer period, oxygen was continuously incorporated into the dough and made available to the reaction sites in excess of requirements. In such conditions sulphydryl losses occurred both by direct oxidation, and also through the mediation of oxidised lipids. Thus, the loss of sulphydryl groups in defatted flour doughs will be less than in original flour doughs. The addition of oxidised flour lipids, and oxidised linoleate, to nitrogen mixed doughs brought about a decrease in sulphydryl content, and an improvement effect.

The conclusions of Tsen and Hlynka (137,139) have been challenged by Dahle and Sullivan (128). The latter workers were unable to demonstrate the oxidation of glutathione by a wheat lipoxidase-linoleate system. By assay of oxidised lipids formed during dough mixing a slight interaction between sulphydryl groups and oxidised lipids was established, however the reaction was considered to be unimportant unless the mixing was long or accomplished in oxygen. The conclusion

was reached that fatty acid peroxides do not oxidise sulphydryl groups during mixing to any significant degree, and that such a reaction would only be a minor factor of maturing action during mixing.

Bloksma (140) found that the removal of flour lipids decreased the rate of sulphydryl oxidation in doughs, in direct contradiction to the observations of Tsen and Hlynka (139). Thus, the reaction of sulphydryl groups with fatty acid peroxides must be considered of problematical significance. Bloksma considered that the higher amount of peroxides found in the presence of sulphydryl blocking agents could be better explained by the failure of the sulphydryl-peroxide reaction, rather than an increase in lipid oxidation. This explanation, however, is not in accord with the observations of Dahle and Sullivan (128).

To conclude this review the following quotation from the paper of Dahle and Sullivan (128) indicates the need for further research:-

> 'Although much has been learned in recent years about the various mechanisms responsible for flour improvement, further work is needed to evaluate the relative importance of lipid and other oxidising systems and their effect on the -SH -S-S- interchange and subsequent dough properties.'

EXPERIMENTAL

EXPERIMENTAL

PART 1 - FOREWORD

Minute amounts of oxidising agents are known to have disproportionate, and when properly controlled, beneficial effects in modifying the properties of dough made from wheat flour. The implication of atmospheric oxygen in this 'improvement process' has been demonstrated by various workers (8,9,53,54,81,82). These studies were followed by the patenting of the Rank and Hay 'batter' process (2), and the oxygen process of Todd, Hawthorn and Blain (98). Both of these processes relied on the incorporation of air, or oxygen, into a flour batter during high speed mixing to achieve a bleaching and improving effect.

Manometric devices have been used to measure the amount of oxygen taken up by wheat flour when it is wetted (10,11). The apparatus and methods possessed certain disadvantages when rapid and accurate measurements of small amounts of oxygen were required.

These disadvantages were:-

(1) A general lack of sensitivity to small changes in oxygen pressure or partial pressure.

(2) The rate of attainment of equilibrium between the gas and liquid phase was a limiting factor when studying the rate of oxygen uptake.

In order to obtain a rapid measurement of the oxygen uptake rate in a suspension, a highly sensitive method for continuously recording the concentration of dissolved oxygen is required.

The polarographic method for oxygen determination is suited to such a case, and this technique was adopted to obtain the results presented in this thesis.

Theory Underlying the Polarographic Determination of Oxygen

In 1924, Heyrovsky (141) reported that oxygen is reduced at the dropping mercury electrode, the polarogram consisting of two waves of equal height (Fig. 1).

The first wave results from the reduction of oxygen to hydrogen peroxide. The second wave corresponds to the reduction of the hydrogen peroxide to water or hydroxyl ions, depending on the pH (142). The value of the plateau height (A,Fig.1) on the current axis is known as the diffusion current for oxygen. The value of the diffusion current depends on the rate of diffusion of oxygen to the mercury surface, and is directly related to the concentration of dissolved oxygen in the electrolyte. Thus, by applying a voltage in the range -0.5 volts to -0.7 volts versus the saturated calomel electrode (S.C.E.) and noting the current on a sensitive ammeter, it is possible to observe changes in the dissolved oxygen concentration. Expressed in mathematical terms the current:oxygen concentration relationship is:-

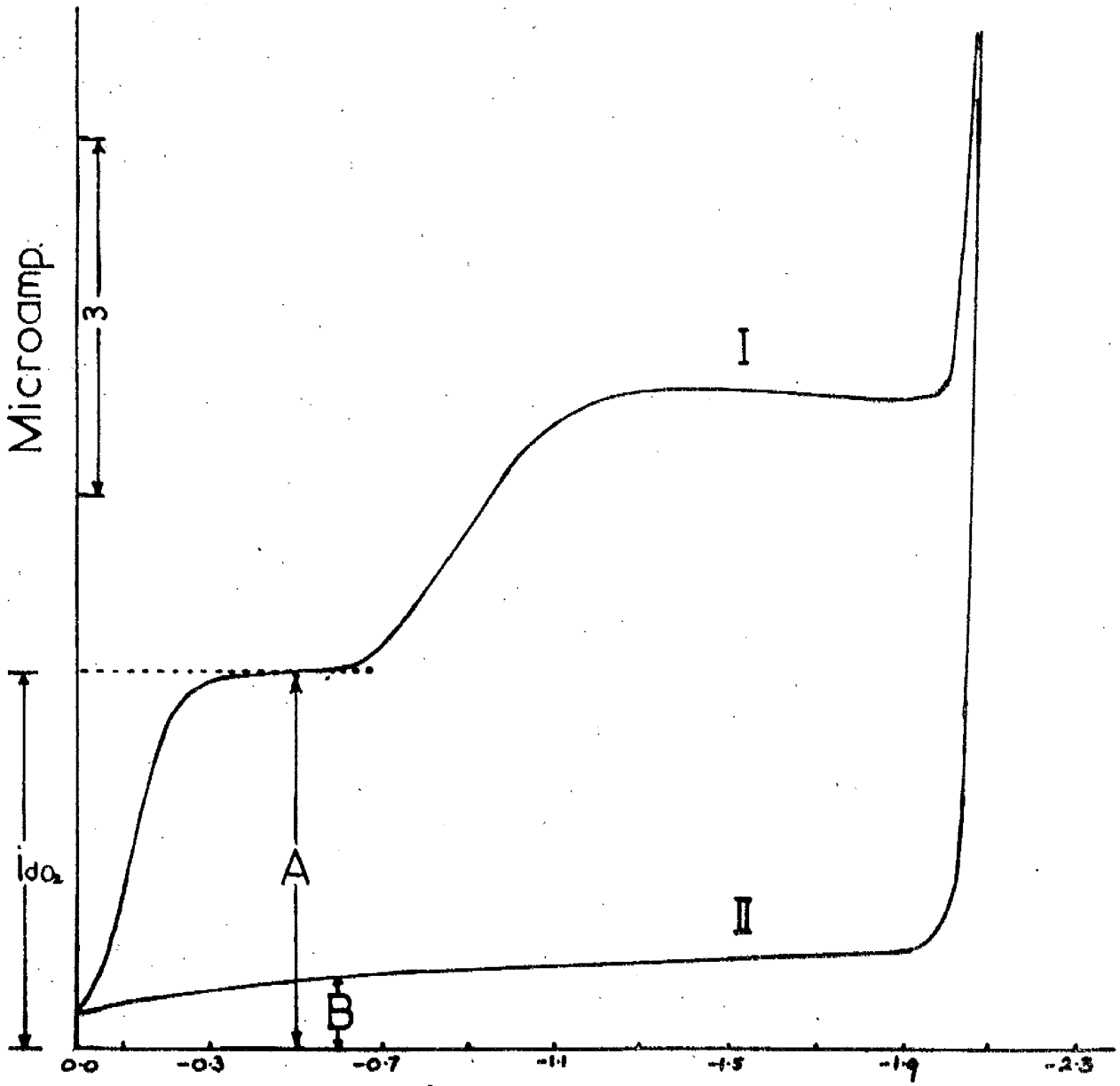
$$i_d = k [O_2]$$

where i_d = diffusion current for oxygen, and k is a constant.

Figure 1

Polarogram of Oxygen Obtained with the Dropping Mercury Electrode (DME)

(after Kolthoff & Lingane[161])



I 0·05M. Potassium Chloride, Air Saturated + Trace of Methyl Red

II Residual Current after Removal of Air by Nitrogen

This method, for the determination of dissolved oxygen, was first used by Vitek (143,144) who confirmed that the diffusion current was linearly related to the concentration of dissolved oxygen in various organic solvents.

Certain precautions have to be observed when this method is employed. It is necessary to suppress the oxygen maximum, which is observed in weak electrolyte solutions of less than 0.1 M. in strength, and which will distort the shape of the first plateau. The oxygen maximum may be eliminated by traces of such substances as gelatin, dye stuffs and protein (145). Secondly, it is essential to ensure that no other material is present which may interfere in the determination of oxygen. The presence of such a substance is easily detected by bubbling nitrogen through the test solution until the current reading becomes steady. If only oxygen is being reduced the current reading will be zero or of negligible value (the residual current, B,Fig.1). The value of the residual current will be appreciable in the case of an interfering substance, and must be deducted when calculating the diffusion current due to oxygen. Finally, the temperature must be kept constant throughout the determination, as the solubility of oxygen will vary with the temperature of the test solution (146,147).

The first reported application of the polarographic method for the determination of dissolved oxygen in biological fluids was by Baumberger and Müller (148), in 1935. In 1938, Petering and

Daniels (149) gave details of a polarographic method for studying the respiration of yeast, algae and red blood cells. Since its initial application, numerous workers have employed this method successfully (150 - 156).

The Polarographic Determination of Oxygen with the Rotating Platinum Electrode (R.P.E.)

A later development of the polarographic technique for the determination of oxygen came with the introduction of the rotating platinum microelectrode by Kolthoff and Laitinen (157,158). These workers demonstrated the greatly increased oxygen diffusion current with this electrode, thus enhancing the sensitivity of the method. Giguère and Lauzier (152) reported that the value of the diffusion current had increased by a factor of fourteen times using this electrode, compared with the dropping mercury electrode. The use of this electrode is further commended by the fact that the solution being studied can be stirred without causing fluctuations in the current readings. The use of the dropping mercury electrode has been questioned when employed for the study of biological systems, due to the toxic action of mercuric ions (159,160).

The reduction of oxygen at the rotating platinum microelectrode has been discussed by Kolthoff and Lingane (161). The theoretical basis of the determination of the dissolved oxygen is essentially similar to that involved when the dropping mercury

electrode is used, and may be stated as follows:-

By applying a gradually increasing potential across the electrodes oxygen is reduced at the cathode, and a current flows which is proportional to the rate of reduction. The rapid rotation of the electrode results in the retention of a very thin layer of stationary liquid (diffusion layer) around the moving part, whilst the bulk of the solution is fairly uniformly mixed. If a sufficiently great potential difference is applied to reduce all the oxygen at the platinum surface, a diffusion gradient will be set up across the stationary layer. The rate at which oxygen diffuses through the layer will be proportional to its concentration in the bulk of the solution, thus the current flowing through the cell will be proportional to the concentration of oxygen in solution.

The polarogram of oxygen obtained with a rotating platinum electrode shows a similar first wave to that found with the mercury electrode (Fig.2).

The second wave is difficult to observe as the reduction curve of oxygen becomes very elongated (152).

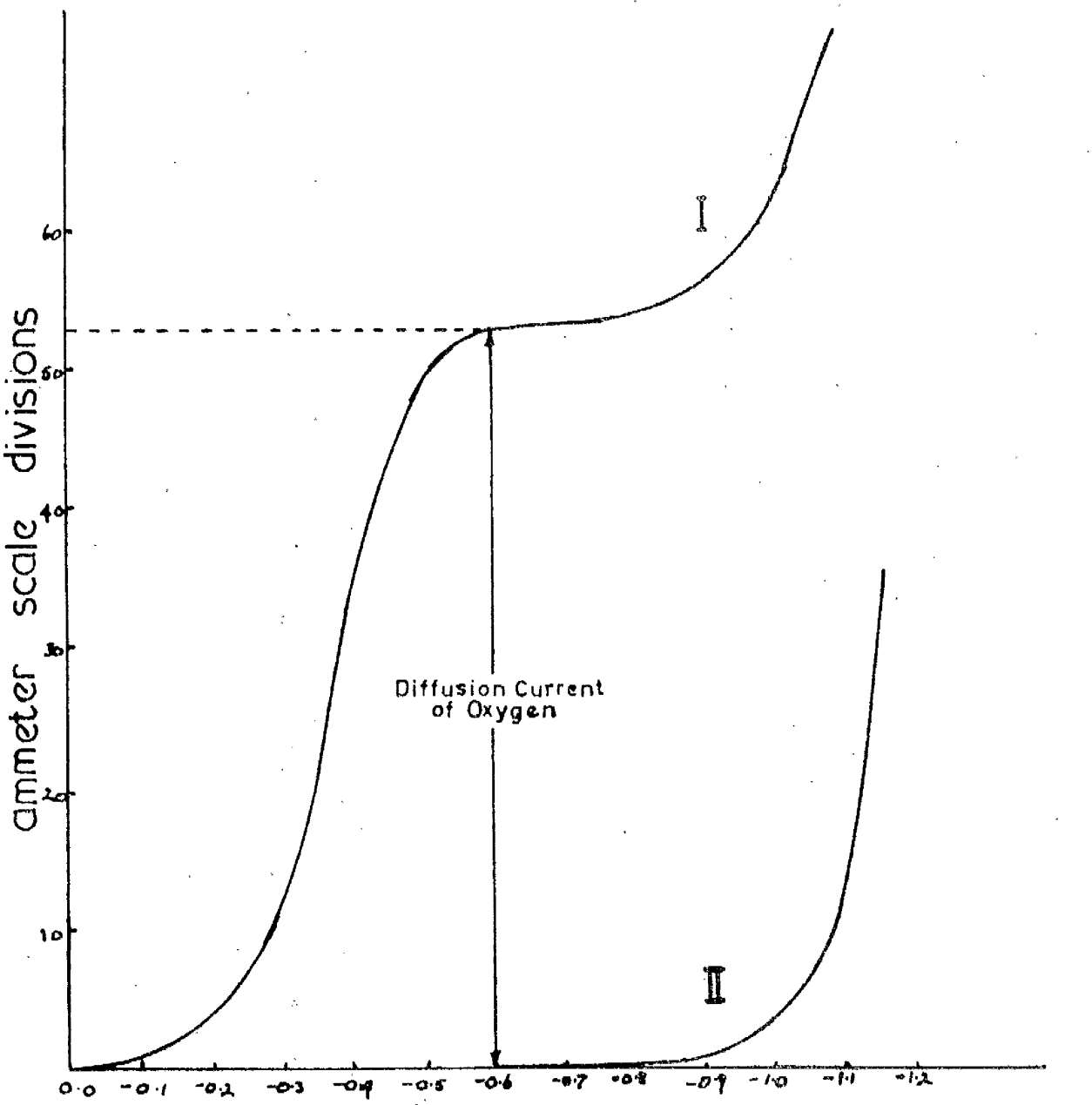
The electrode reaction for the reduction of oxygen at a platinum surface, according to Kolthoff and Lingane (161) is:-

$$O_2 + 2H_2O + 2e \longrightarrow H_2O_2 + 2OH^-$$

When determining oxygen amperometrically with the R.P.E. a suitable negative potential, referred to a standard half cell

Figure 2

Polarogram of Oxygen Obtained with the Rotating Platinum Electrode (R.P.E.)



I 0·125M. Potassium Chloride Air Saturated

II Residual Current after Removal of Oxygen by Addition of Sodium Sulphite

e.g. the saturated calomel or silver/silver chloride electrode, should be chosen. The selection of this voltage is dependent on the diffusion current of oxygen, which is observed between -0.6 volts and -0.9 volts vs. S.C.E. (plateau region of the polarogram Fig.2), and which is linearly related to the oxygen concentration. Steel and Brierley (162), and Warshowsky and Schantz (159) used an applied voltage of -0.75 volts vs. S.C.E. when studying aeration in submerged fermentations, whilst Marsh (163) employed a voltage of -0.7 volts vs. S.C.E. in determining the dissolved oxygen content of oil field brines.

Calibration of the Recording System

The polaregraphic method for the determination of dissolved oxygen relies on recording the diffusion current due to oxygen at a fixed negative potential. The value of the diffusion current may be related to a particular oxygen concentration by prior calibration of the ammeter scale in the appropriate units.

The procedure, adopted by various workers (149,152,154,159, 162,163), is to determine the dissolved oxygen concentration in a suitable electrolyte solution e.g. 0.1 M. KCl by the Winkler method (164). The oxygen diffusion current for this solution is then recorded, and a calibration factor may be derived which relates current to concentration.

Determination of the Uptake of Dissolved Oxygen in Flour Suspensions

Apparatus

The apparatus employed in this study is illustrated on pages 55,56,59, and described under PART 2 of this section.

Method

The general method employed for observing the oxygen uptake of flour suspension is described under PART 3 of this section.

Calibration

The calibration procedure may be found under the Appendix.

EXPERIMENTAL

PART 2 - APPARATUS

The instrument used for the amperometric determination of dissolved oxygen in flour suspensions was a polarograph (Cambridge Instrument Co. Ltd.). The galvanometer was set at a sensitivity of 1/30 when recording the oxygen diffusion current, as at this setting a full scale deflection was obtained with 0.125 M.KCl which was used in preparing the flour suspensions.

The polarograph was connected to a 6 volt. D.C. supply and the voltage applied to the rotating platinum cathode was regulated to -0.6 volts vs. S.C.E. This voltage corresponds to the region of the polarogram of oxygen where the diffusion current is observed (Fig.2 p. 49); therefore the recorded current was proportional to the dissolved oxygen concentration. The residual current, noted after removal of dissolved oxygen from the flour suspensions, was negligible.

The rotating platinum electrode employed for this study was similar in design to that of Laitinen and Kolthoff (158). The electrode consisted of a platinum wire 3.2 mm. long and 0.5 mm. in diameter set into a $\frac{1}{4}$" diameter iron shaft. The platinum wire was protected from damage by flanges on the iron shaft set $\frac{1}{8}$" above and below the wire. The iron shaft was mounted in

brass bearings incorporating a mercury seal device. The assembly was carried in a large rubber bung which was also bored to accommodate the saturated calomel electrode. A calomel electrode with a sintered glass plug (Cambridge Instrument Co. Ltd.) was found to be satisfactory because it showed high stability and was easily cleaned.

The platinum electrode was rotated at a speed of 600 r.p.m. through a 2.5:1 gear reduction from a 1/20 h.p. synchronous speed motor (Metropolitan-Vickers Electrical Co. Ltd.). This speed of rotation has been established as suitable for the determination of oxygen (152,158). The use of a synchronous speed motor is essential in order to obtain reproducible results, as the speed of rotation will influence the magnitude of the diffusion current. It was necessary to provide the gear system with insulating bushes to prevent any stray current from the motor interfering in the observation of the diffusion current.

The iron shaft of the electrode was painted with cellulose enamel, and subsequently coated with a layer of ceresin wax. This treatment ensured that the platinum wire was the only charged metal surface in contact with the solution; further it prevented rusting of the shaft. A perspex splash-disc was fitted to the shaft at the point where it entered the rubber bung, thus preventing traces of moisture reaching the brass bearings.

An inverted steel sleeve ($\frac{3}{4}$" diameter) was mounted on the iron shaft, so that its open end could rotate in the mercury seal. By this means direct electrical contact was established between the platinum wire and a brass terminal on the electrode housing. Both electrodes were connected directly to the polarograph. The electrode assembly is shown in Fig. 3. p. 55.

The temperature of the 0.125 M.KCl solution used to make up the suspension was standardised at 25° ± 0.2° C. by immersion in a water bath, the temperature of which was maintained by a circulating thermostatic heater (Circotherm, Shandon Scientific Co. Ltd.). An air pump (Evans Electroselenium Ltd.) was used to bubble a slow stream of humidified air through the solution, thus ensuring a standard initial oxygen tension in the suspension.

The suspension was prepared by mixing the 0.125 M.KCl solution with a weighed quantity of flour, for 1 min., in a high speed macerator (Townson and Mercer Ltd.) Fig. 4. p. 56.

A graduated 250 ml. polythene beaker was used as the polarographic cell. A sample of the suspension, contained in this cell, was transferred to a water bath consisting of a large polythene beaker fitted with a perspex flange to support the cell. The water bath was maintained at 25° C. by water which was pumped from a main tank by the circulating pump on the thermostat. The water was returned to the main tank by an overflow tube which was inserted into the side of the bath. The water bath was enclosed

Fig. 3.

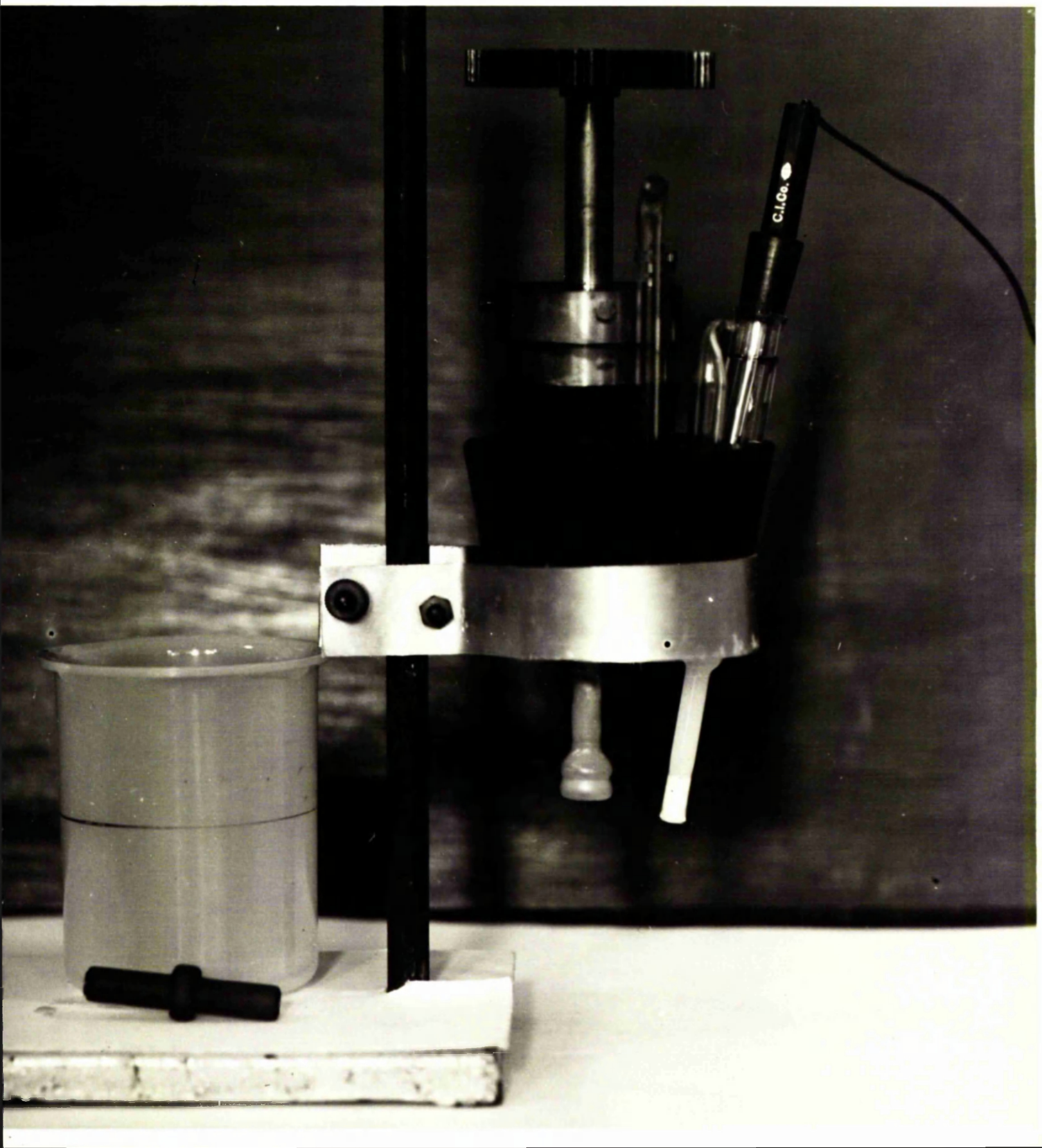
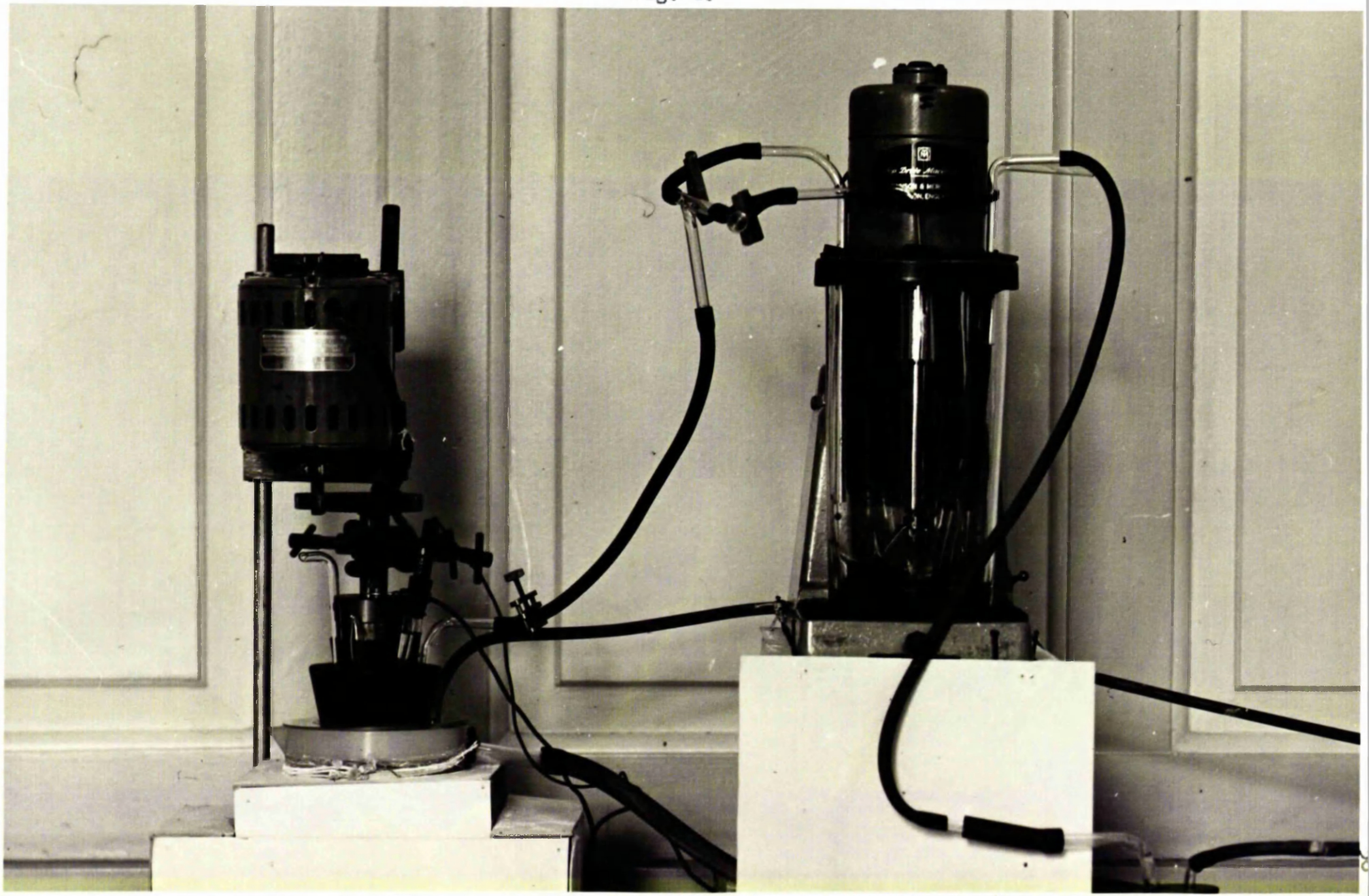


Fig. 4.



in an internally lagged box, in such a fashion that its base rested on the housing of an electrically operated rotating magnet. This magnet gently rotated a rubber-covered iron stirrer on the bottom of the cell.

An accurate stop-watch was used to take readings of the diffusion current, at 30 sec. intervals, over a period of 20 min.

The general arrangement of the complete apparatus is shown in Fig. 5 p. 59.

Fig. 5.

General Arrangement of Apparatus

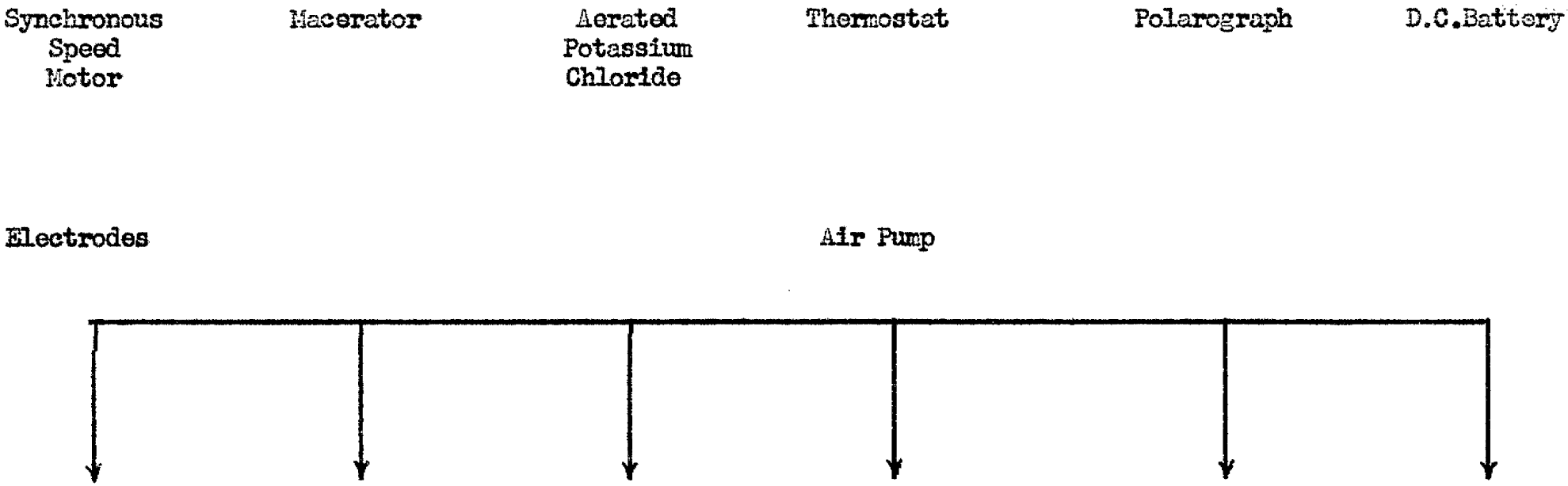
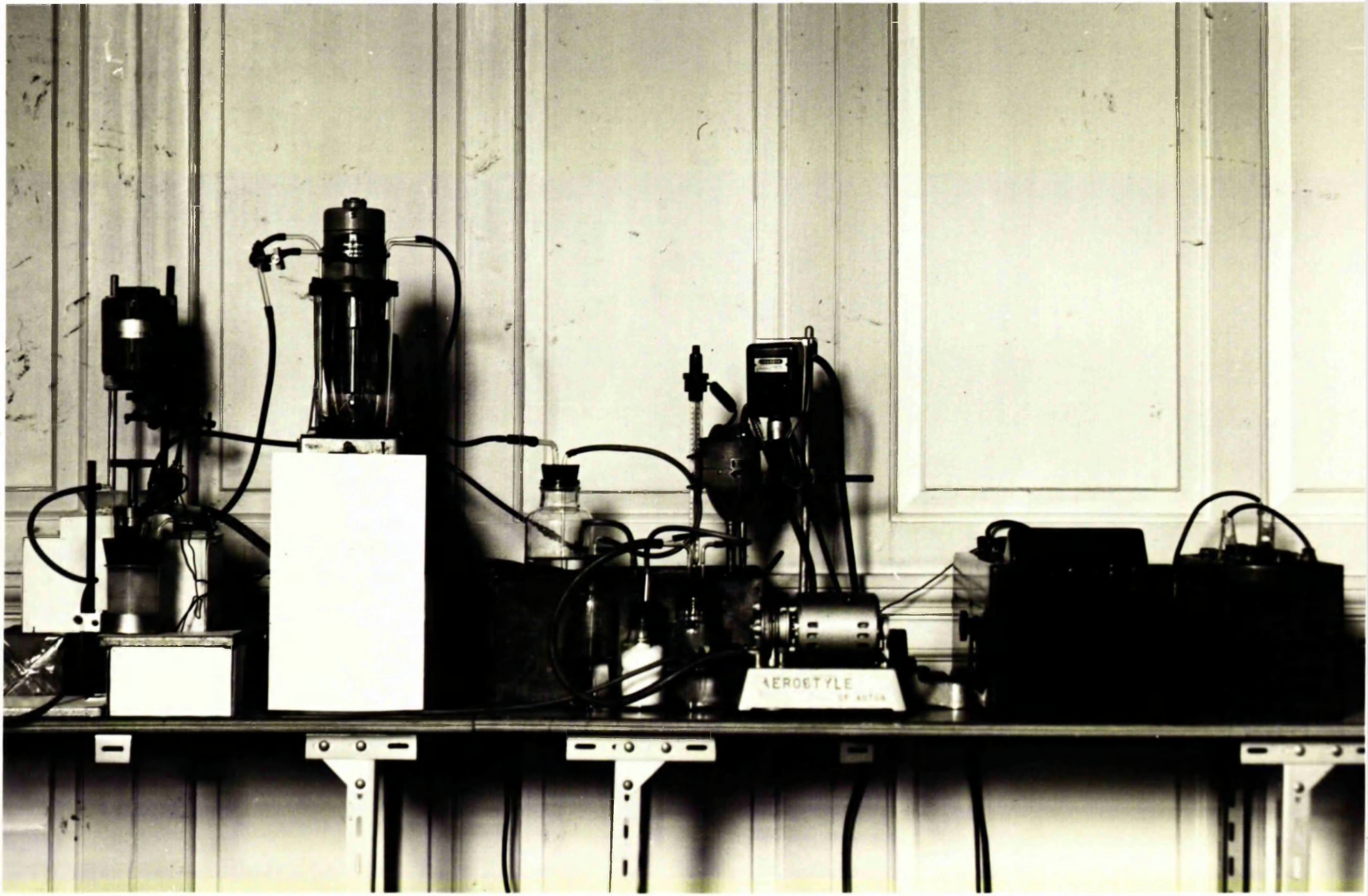


Fig. 5.



EXPERIMENTAL

PART 3 - METHOD

The solution used to make up the suspension was 0.125 M. potassium chloride. This solution supplied dissolved oxygen to the flour in suspension, its oxygen content being 6.18 ml./litre at 25° C., as determined by the Winkler technique (164).

450 ml. of 0.125 M.KCl were made up from 50 ml. 1.125 M.KCl ('Analar') stock solution using deionised water. The solution, in a 1 litre glass jar, was placed in the thermostat bath for a 30 min. period and allowed to attain a temperature of 25° C. A controlled stream of air was bubbled through the solution during this period in order to ensure that the oxygen content was standardised for each experiment. The air was supplied from the air-pump and was led through a filter and humidifiers, to prevent any evaporation from the solution during the oxygen and temperature equilibration period.

18g. of flour were placed in the mixing vessel of the macerator. The 0.125 M.KCl was transferred rapidly to this vessel through a side arm in the lid of the macerator. The flour and the solution were thoroughly mixed for 1 min., which was found to be the minimum time that could be allowed to ensure complete wetting of the flour. The suspension was withdrawn by siphoning through a second side arm, via

a rubber tube, into the graduated 250 ml. polythene beaker which served as the polarographic cell. The siphoning action could be conveniently started by using a connection attached to the air inlet of the pump. The electrode system, mounted in a rubber bung, had previously been inserted into the cell, together with a rubber covered magnetic stirrer. When 150 ml. of the suspension had been transferred to the beaker, the siphoning action was stopped by tightening a screw clip on the rubber tube. The complete assembly was placed in a second thermostatically controlled water bath, which contained water circulating at 25°C., pumped from the main bath. The rotating electrode was clamped in position, so that the gear wheel attached to the electrode engaged the gear wheel fixed to the synchronous speed motor. The magnetic stirrer and motor were started simultaneously and the electrodes connected to the polarograph. The speed of rotation of the electrode was constant at 600 r.p.m.

A voltage of -0.6 volts vs. S.C.E. was supplied to the rotating platinum cathode. The diffusion current due to oxygen was observed over a period of 20 min., at 30 sec. intervals, on a moving-spot galvanometer. A galvanometer sensitivity of 1/30 was found to be satisfactory for recording the oxygen uptake of the suspension.

The oxygen maximum, which is observed in pure dilute electrolytes was not observed in the presence of flour, so no suppressor was required.

It was found that oxygen was the only substance present in the suspension which would undergo reduction at an applied voltage of -0.6 volts vs. S.C.E. The residual current, observed when oxygen had been removed from the suspension by addition of sodium sulphite or by a stream of nitrogen, was negligible. The diffusion current of oxygen was not influenced by the gentle stirring action of the magnetic stirrer.

In several of the initial experiments the air space over the surface of the suspension was replaced by a stream of nitrogen. Over the experimental period no difference in the results could be detected, indicating that oxygen was being removed from the suspension at a faster rate than it could diffuse in from the atmosphere.

Good reproducibility could be obtained by scrupulously cleaning both electrodes after each experiment.

The flour was stored in large glass jar of about 4 lb. capacity at a temperature of + 4° C. Storage over a period of three weeks did not significantly affect the oxygen uptake of the flour.

Overall Experimental Reproducibility

With practice the galvanometer could be read to the nearest ½ of 1 scale division, which corresponds to ± 4.4 µl. of dissolved oxygen. As there are initially 927.1 µl. of dissolved oxygen in the suspension, the high sensitivity of the method may be appreciated.

Each experiment was performed in triplicate, the deviation from the mean uptake curve, for an individual experiment, being as follows:-

(1) Standard deviation from the mean of reading at 5 min. after wetting is ± 1.96 scale divisions.

(2) Standard deviation from the mean of reading at 20 min. after wetting is ± 1.18 scale devisions.

As the reading at 0 time is constant in each experiment the deviations may be reported in terms of µl.O_2. thus:-

(1) Standard deviation in uptake 0-5 min. is ± 34.5 µl.O_2.

(2) Standard deviation in uptake 0-20 min. is ± 20.8 µl.O_2.

The difference in the standard deviations is due to the fact that the initial uptake of oxygen by the flour suspension is very rapid, consequently the moving-spot galvanometer is difficult to read accurately. After 20 min. the oxygen absorption is much slower and generally tends to a constant level for each flour, thus increasing the reproducibility of the observation.

The standard deviations were calculated by the usual statistical methods, and were based on 78 experimental observations.

RESULTS

RESULTS

PART 1

The Oxygen Uptake of Untreated Flour Suspensions

A. Samples Studied

Nine samples of Spring flour were obtained in treated and untreated forms. The designations given to these flours were:-

Spring Flours:- Series 1 - Series 9.

Thus, Spring Flour Series 1 refers to either a treated or untreated sample of this particular flour. The treatment if any, and its nature, is indicated in each TABLE.

B. Apparatus and Method

The concentration of dissolved oxygen in the flour suspensions was followed by a sensitive polarographic technique. Full details of the apparatus and method will be found under PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p.52 and 60 respectively.

C. Results

The oxygen concentration of the suspensions was recorded over the range 3 min. 45 sec. to 20 min. after the flour had been initially wetted (zero time). A typical experimental report is presented below.

Oxygen Uptake of a Flour Suspension with Respect to Time

EXPERIMENTAL REPORT

Flour Series:- 2 Run No.:- 2

Supporting Electrolyte:- 0.125 M.KCl

Volume of Suspension Studied:- 150 ml. (≡ 6g. flour).

Temperature of Suspension:- 25° C.

Applied Voltage:- -0.6v., relative to a standard calomel electrode.

Galvanometer Sensitivity:- $\frac{1}{30}$

Rotational Speed of Platinum Microelectrode:- 600 r.p.m.

Initial Oxygen Concentration:- 927 µl. O_2. (≡ 52.6 scale divisions.)

Time from wetting of flour (min.)	Polarograph Scale Divisions[a]	Time from wetting of flour (min.)	Polarograph Scale Divisions[a]
3.75	32.00	12.0	15.25
4.00	29.75	12.5	15.00
4.25	27.00	13.0	14.50
4.5	26.25	13.5	14.25
5.0	25.00	14.0	14.00
5.5	23.75	14.5	13.75
6.0	22.75	15.0	13.50
6.5	21.75	15.5	13.25
7.0	21.00	16.0	13.00
7.5	20.25	16.5	12.75
8.0	19.50	17.0	12.75
8.5	18.75	17.5	12.50
9.0	18.00	18.0	12.25
9.5	17.50	18.5	12.00
10.0	17.00	19.0	12.00
10.5	16.50	19.5	11.75
11.0	16.00	20.0	11.75
11.5	15.75		

[a] 1 Scale division ≡ 17.62 µl. O_2.

The experiment was conducted in triplicate for each flour, and the mean curve of dissolved oxygen concentration against time was plotted. A typical graph showing the oxygen uptake of a flour suspension is illustrated on p.75 Diagram 1 (Flour Series 2 Untreated).

All graphs (Diagrams 1-14) are drawn on common ordinates for convenience of comparison. The graphs only record observations from 5-20 min. since it was not possible to observe the detailed shape of the curves between 0-5 min. It should be noted that the reading of oxygen concentration at zero time is 52.6 scale divisions in all cases.

It was found that no simple mathematical relationship could adequately represent the varied forms of the uptake curves. This being the case the results are expressed throughout in the manner of TABLE 1.

General Note

The results reported in PARTS 1-8 represent the collected data from the various experiments comprising this study. The significance of these results is commented on in the DISCUSSION (p.112).

TABLE 1.

Oxygen Uptake by Untreated Flour Suspensions

Flour	(a) Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	(a) Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Series 1	620	215	139	90
Series 2	469	270	123	80
Series 3	418	354	129	83
Series 4	227	372	99.9	65
Series 5	507	242	125	81
Series 6	557	215	129	83
Series 7	372	143	85.8	56
Series 8	615	90	118	76
Series 9	441	264	118	76

(a) Oxygen Uptakes are reported on a 6g. flour basis in TABLES 1-24, unless otherwise stated.

PART 2

The Oxygen Uptake of Treated Flour Suspensions

A. Samples Studied

A Series of seven Spring flours were used to study the effect of commercial bleaching and improving agents on the oxygen uptake process. The samples were treated at the following levels:-

Flour Series 1

(a) Benzoyl Peroxide.....1 oz./sack (280lb.), diluted with carrier ≡ 33 p.p.m. benzoyl peroxide.

(b) Chlorine Dioxide.....1.5g./sack ≡ 12.0 p.p.m. chlorine dioxide.

(c) Potassium Bromate....0.75./sack ≡ 6.0 p.p.m. potassium bromate.

(d) Fully Treated.....Benzoyl peroxide, chlorine dioxide and potassium bromate at the above levels.

Flour Series 2

(a) Benzoyl Peroxide.....1.75g./sack, diluted with carrier ≡ 58 p.p.m. benzoyl peroxide.

+ (b) Potassium Bromate..7.5g./sack, 10% strength, ≡ 6.0 p.p.m. potassium bromate.

Flour Series 3-7

As for Flour Series 2.

B. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p. 52 and p. 60 respectively.

C. Results

The results are reported in TABLES 2-8. A typical graph showing the effect of treatment on the oxygen uptake process is illustrated on p. 75 Diagram 1. The results obtained for Flour Series 1 are illustrated on p. 76 and p. 77, Diagrams 2 and 3.

TABLE 2.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 1	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	620	215	139	90
Benzoyl Peroxide	585	92	113	73
Chlorine Dioxide	596	88	114	74
Potassium Bromate	554	84	106	69
Fully Treated	583	88	112	72

TABLE 3.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 2	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	469	270	123	80
Benzoyl Peroxide + Potassium Bromate	391	135	87.7	57

TABLE 4.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 3	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	418	354	129	83
Benzoyl Peroxide + Potassium Bromate	314	162	79.3	51

TABLE 5.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 4	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	227	372	99.9	65
Benzoyl Peroxide + Potassium Bromate	277	253	88.3	57

TABLE 6.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 5	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	507	242	125	81
Benzoyl Peroxide + Potassium Bromate	511	136	108	70

TABLE 7.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 6	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	557	215	129	83
Benzoyl Peroxide + Potassium Bromate	502	88	98.3	64

TABLE 8.

Oxygen Uptake by Treated Flour Suspensions

Flour Series 7	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	372	143	85.8	56
Benzoyl Peroxide + Potassium Bromate	340	132	78.8	51

Diagram 1

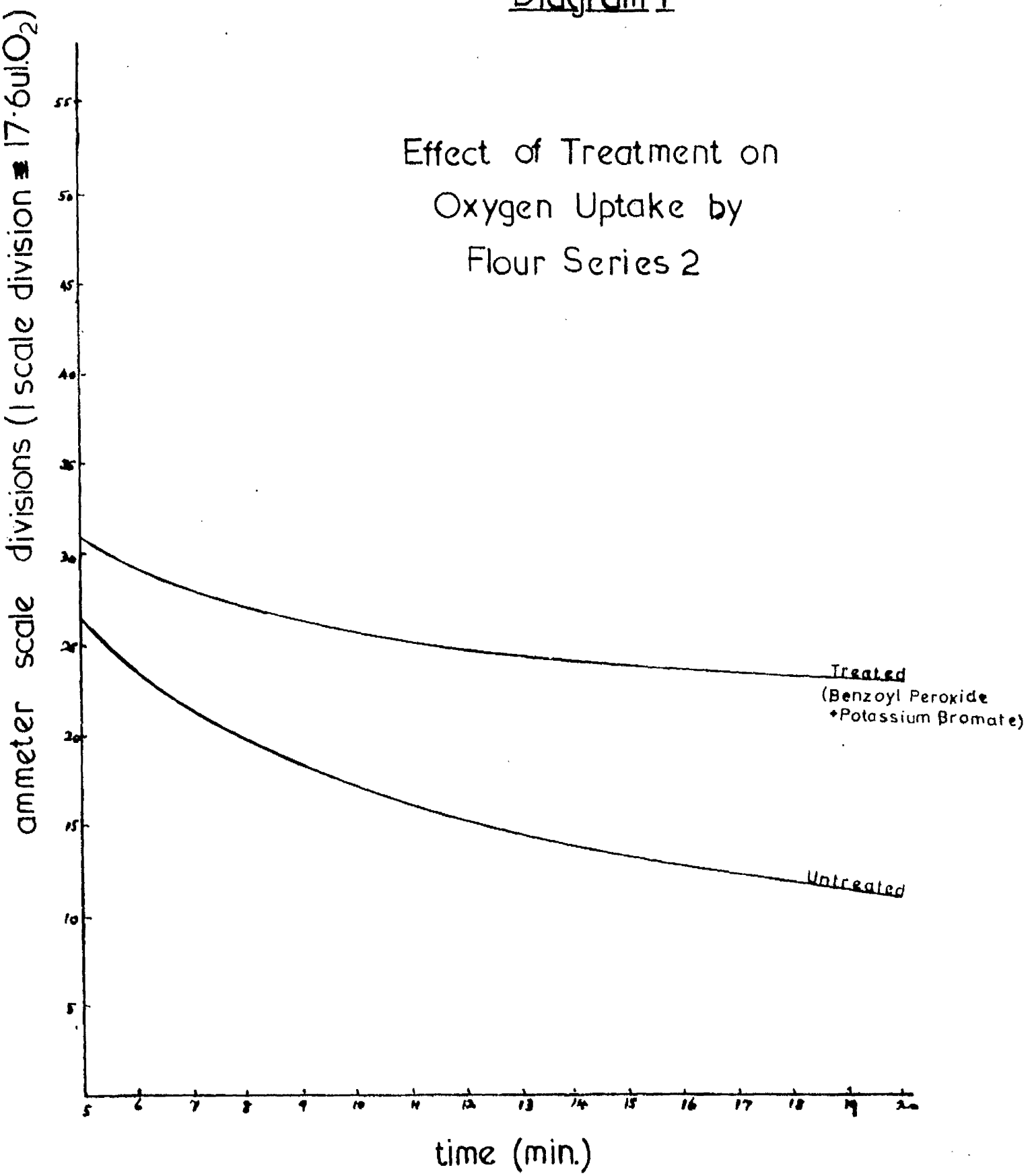

Effect of Treatment on
Oxygen Uptake by
Flour Series 2
ammeter scale divisions (1 scale division ≡ 17·6ul.O2)
55
50
45
40
35
30
25
20
15
10
5
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
time (min.)
Treated
(Benzoyl Peroxide
+Potassium Bromate)
Untreated

Diagram 2

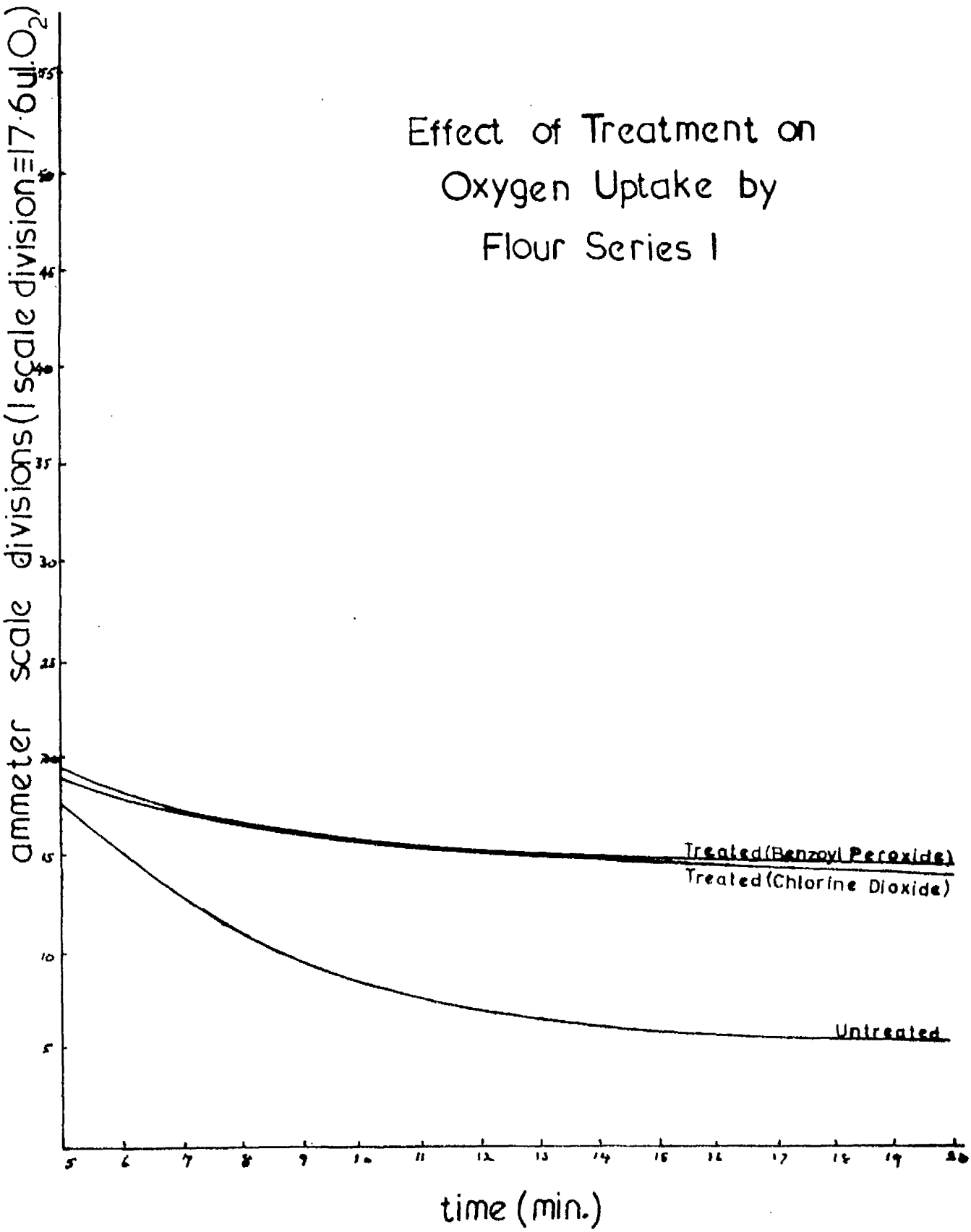
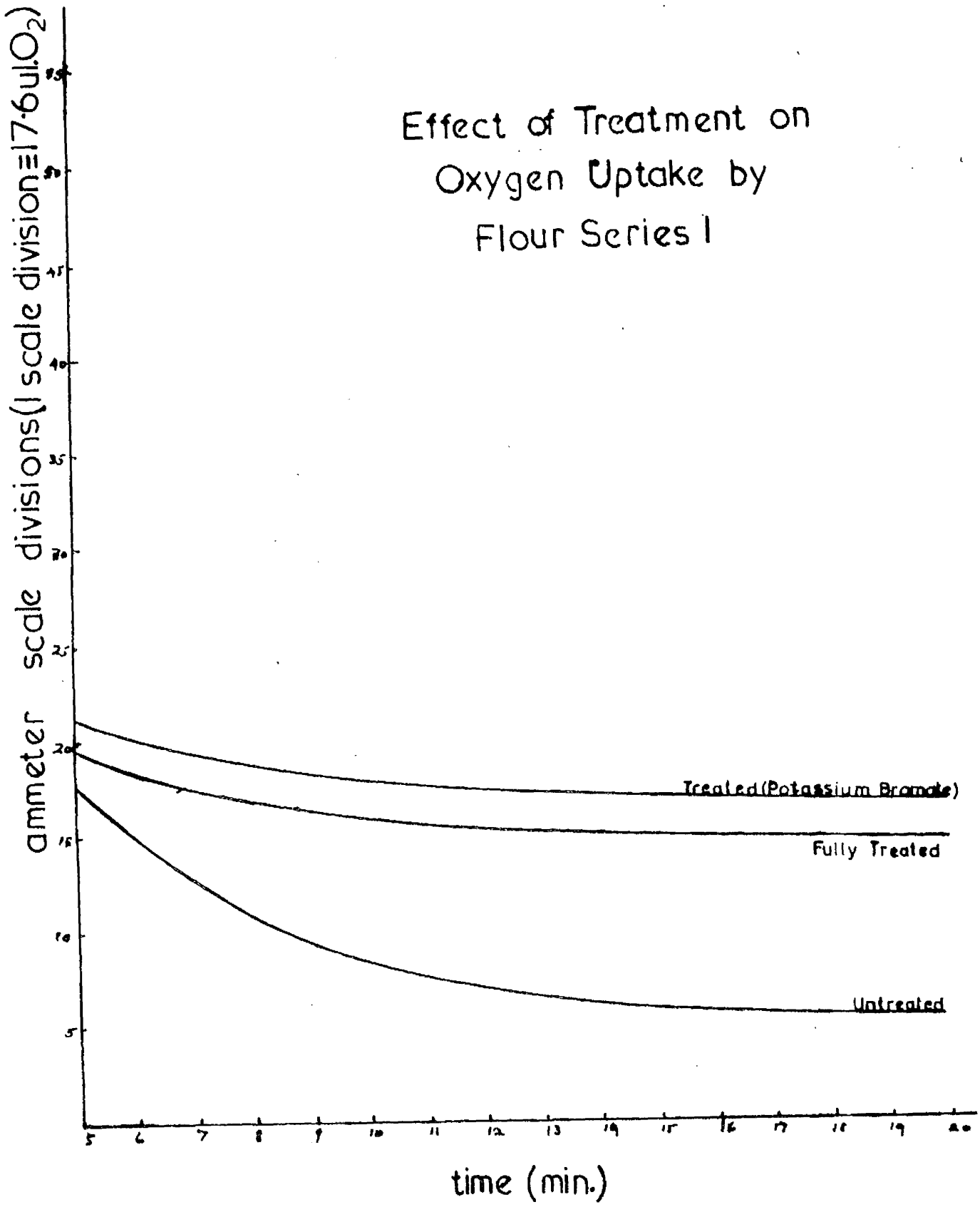

Effect of Treatment on
Oxygen Uptake by
Flour Series I
ammeter scale divisions (1 scale division = 17·6 µl. O_2)
time (min.)
Treated (Benzoyl Peroxide)
Treated (Chlorine Dioxide)
Untreated

Diagram 3


Effect of Treatment on
Oxygen Uptake by
Flour Series I
ammeter scale divisions (1 scale division ≡ 17·6 ul O_2)
Treated (Potassium Bromate)
Fully Treated
Untreated
time (min.)

PART 3

The Oxygen Uptake of Defatted Flour Suspensions

A. Samples Studied

A series of five Spring flours, untreated and treated, were used to determine the effect of fat removal on the oxygen uptake process. The samples were given the following designations

Spring Flour:- Series 1 Untreated

Series 1 Treated with:-

(a) Benzoyl peroxide at a level of 33 p.p.m.

(b) Chlorine dioxide at a level of 12 p.p.m.

(c) Potassium bromate at a level of 6.0 p.p.m.

(d) Fully treated. Benzoyl peroxide, chlorine dioxide and potassium bromate at the above levels.

Spring Flour:- Series 2,3,6,7 Untreated

Series 2,3,6,7 Treated with two agents:-

Benzoyl peroxide at a level of 58 p.p.m.

+ Potassium bromate at a level of 6 p.p.m.

B. Defatting Procedure

1. Petroleum Ether Extraction - Flour Series 1,2,3,

Flour (80g.) was extracted with petroleum ether ("Analar", Boiling range 40° - 60° C.) in a Soxhlet apparatus for a period of 12 hours. The extracted flour was spread out on filter papers so

that the solvent could evaporate at room temperature. The last traces of solvent were removed under reduced pressure in a vacuum chamber connected to an air pump. The flour was stored at 4°C. and allowed to equilibrate with the atmosphere for a period of 2-3 days before being tested.

2. Methanol : Chloroform Extraction - Flour Series 6.7

The method of Morrison (12,165) was followed to remove lipid material. Flour (100g.) was slurried with 200 ml. methyl alcohol ("Analar") in a beaker. The suspension was poured carefully into a percolation column which was closed at the base by a cotton wool plug. The suspension was allowed to settle, most of the methanol running into a receiving flask below the column. 300 ml. of a mixture of methyl alcohol:chloroform (1/1, v./v.) were then gently poured onto the surface of the flour and the solvent allowed to percolate slowly through the flour, removing the lipid material. The flour was freed from solvent as described under 1.

C. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p.52 and p.60 respectively.

D. Results

The results are reported in TABLES 9 - 13. The effects of defatting and of treatment are illustrated by the following graphs:-

TABLE 9

Oxygen Uptake by Defatted Flour Suspensions
(Solvent Petroleum Ether, Boiling range 40° - 60° C.)

Flour Series 1	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.
Untreated	620	215	139	90
Untreated Defatted	231	472	117	76
Benzoyl Peroxide	585	92	113	73
Benzoyl Peroxide Defatted	167	167	55.7	36
Chlorine Dioxide	596	88	114	74
Chlorine Dioxide Defatted	57	238	49.1	32
Potassium Bromate	554	84	106	69
Potassium Bromate Defatted	203	171	62.3	40
Fully Treated	583	88	112	72
Fully Treated Defatted	78	175	42.1	27

TABLE 10.

Oxygen Uptake by Defatted Flour Suspensions
(Solvent Petroleum Ether, Boiling range 40° - 60° C.)

Flour Series 2	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.
Untreated	469	270	123	80
Untreated Defatted	313	431	124	80
Benzoyl Peroxide + Potassium Bromate	391	135	87.7	57
Treated as above Defatted	146	146	48.8	32

TABLE 11.

Oxygen Uptake by Defatted Flour Suspensions
(Solvent Petroleum Ether, Boiling range 40° - 60° C.)

Flour Series 3	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.
Untreated	418	354	129	83
Untreated Defatted	314	428	124	80
Benzoyl Peroxide + Potassium Bromate	314	162	79.3	51
Treated as above Defatted	164	145	51.4	33

TABLE 12.

Oxygen Uptake by Defatted Flour Suspensions
(Solvent Methanol:chloroform 1/1, v./v.)

Flour Series 6	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. 1 g. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.
Untreated	557	215	129	83
Untreated Defatted	357	231	98.0	63
Benzoyl Peroxide ‡ Potassium Bromate	502	88	98.3	64
Treated as above Defatted	258	269	87.9	57

TABLE 13.

Oxygen Uptake by Defatted Flour Suspensions
(Solvent Methanol:chloroform 1/1,v./v.)

Flour Series 7	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.
Untreated	372	143	85.8	56
Untfeated Defatted	85	361	74.3	48
Benzoyl Peroxide ‡ Potassium Bromate	340	132	78.8	51
Treated as above Defatted	95	259	59.0	38

Diagram 4

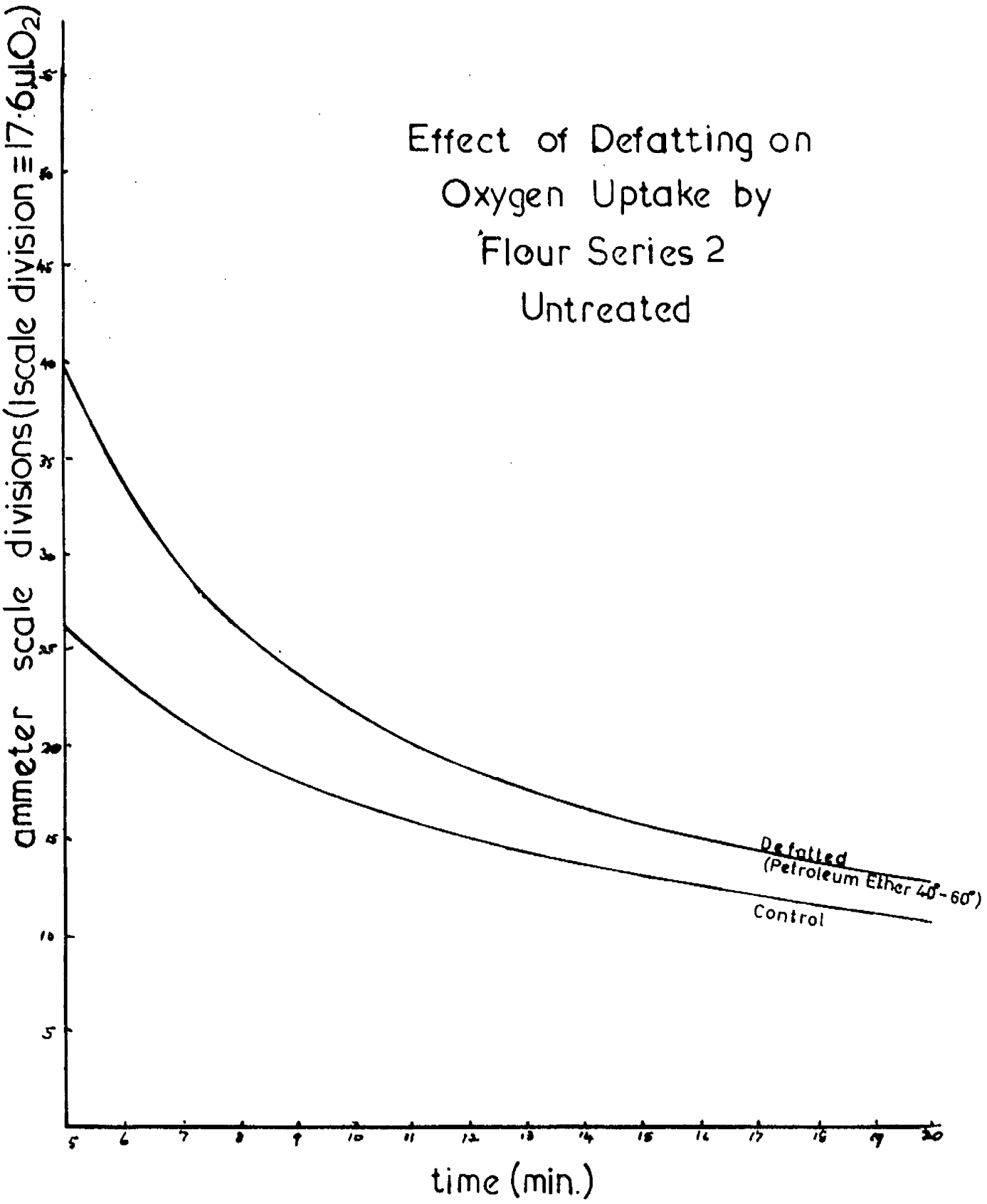

Effect of Defatting on
Oxygen Uptake by
Flour Series 2
Untreated
ammeter scale divisions (1 scale division ≡ 17·6 µl O2)
time (min.)
Defatted
(Petroleum Ether 40°-60°)
Control

Diagram 5

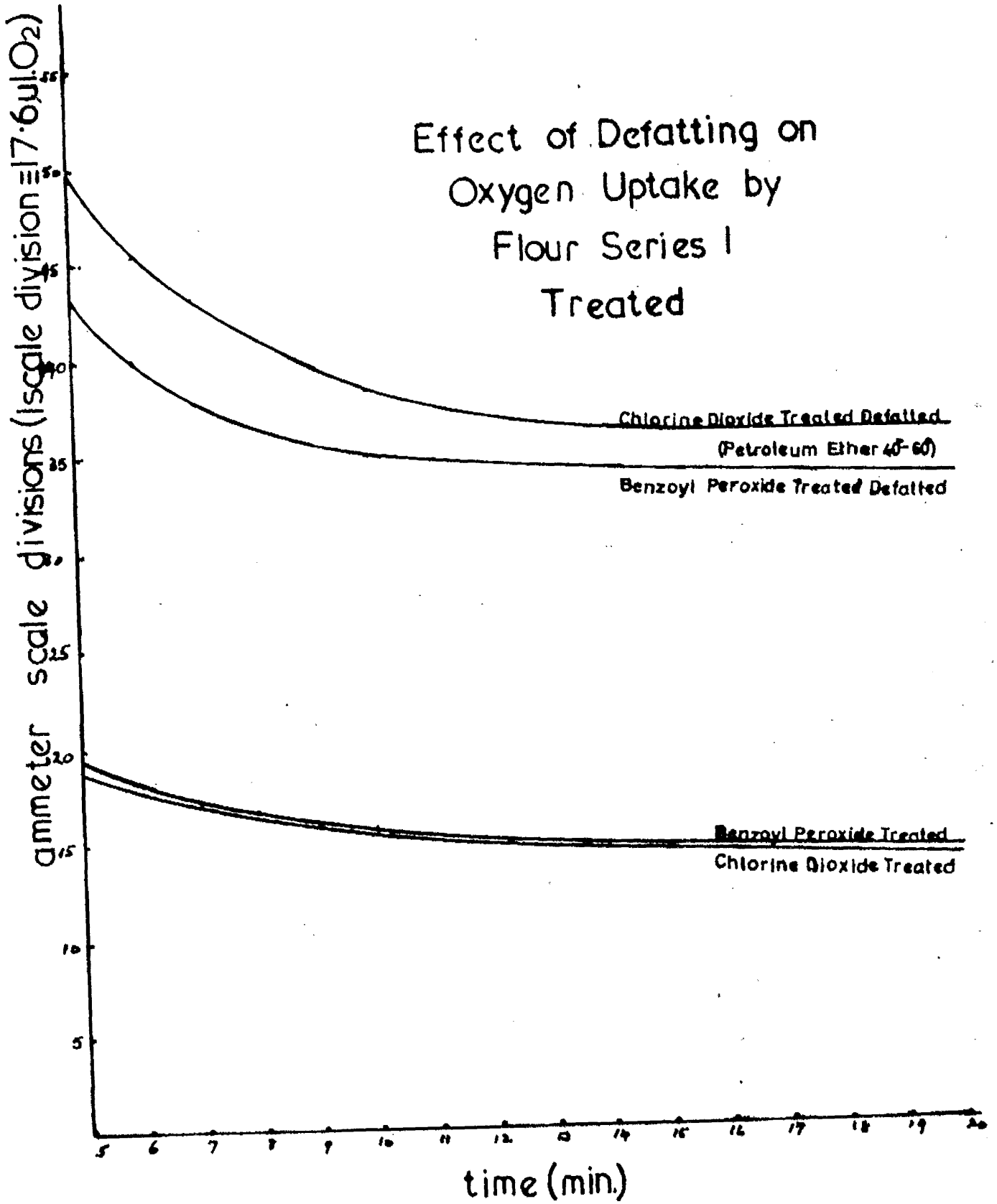

Effect of Defatting on
Oxygen Uptake by
Flour Series I
Treated
ammeter scale divisions (1 scale division ≡ 17·6 µl. O2)
time (min.)
Chlorine Dioxide Treated Defatted
(Petroleum Ether 40°-60°)
Benzoyl Peroxide Treated Defatted
Benzoyl Peroxide Treated
Chlorine Dioxide Treated

Diagram 6

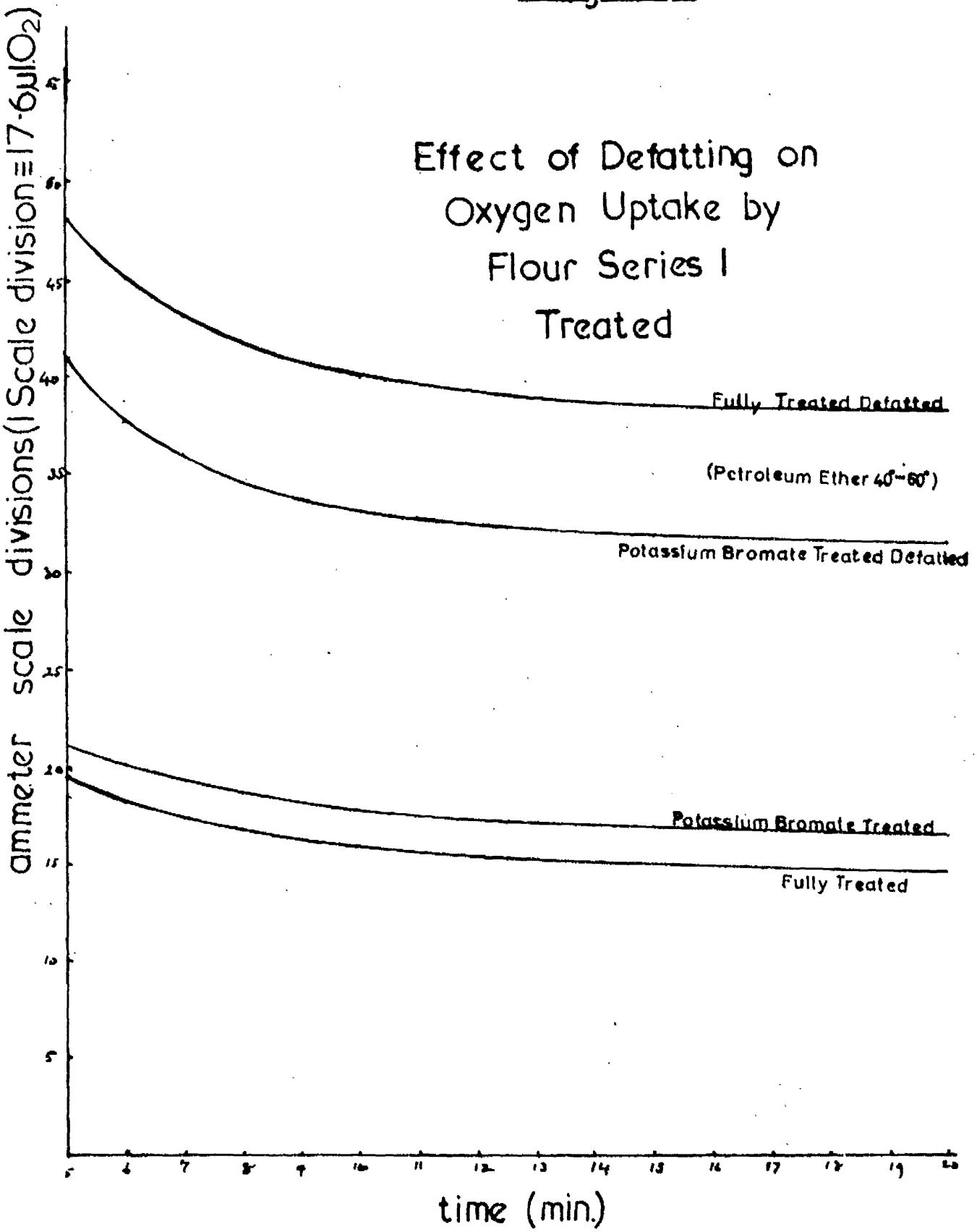

Effect of Defatting on
Oxygen Uptake by
Flour Series I
Treated
Fully Treated Defatted
(Petroleum Ether 40°-60°)
Potassium Bromate Treated Defatted
Potassium Bromate Treated
Fully Treated
ammeter scale divisions (1 Scale division ≡ 17·6 µl O2)
time (min.)

Diagram 7

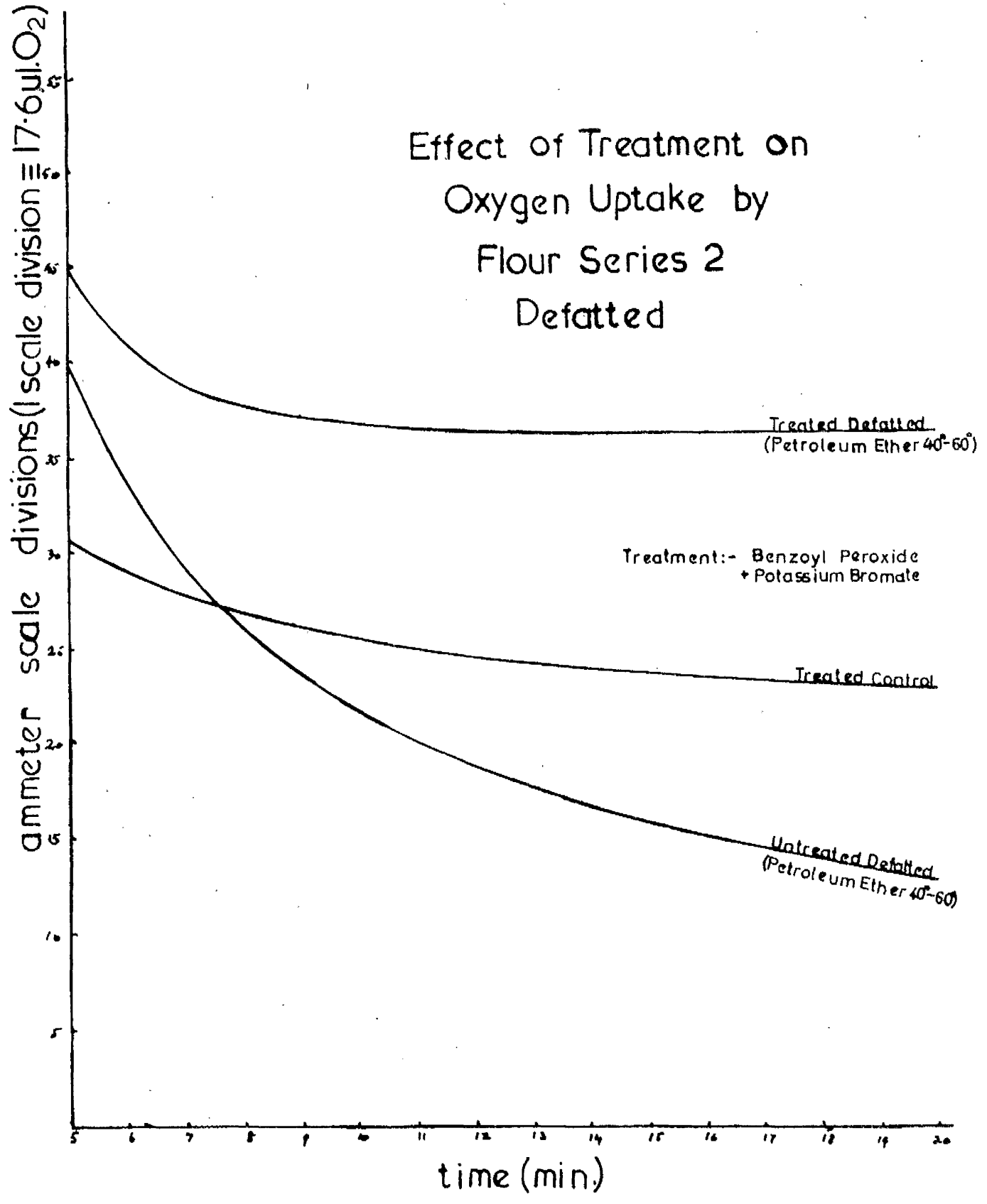
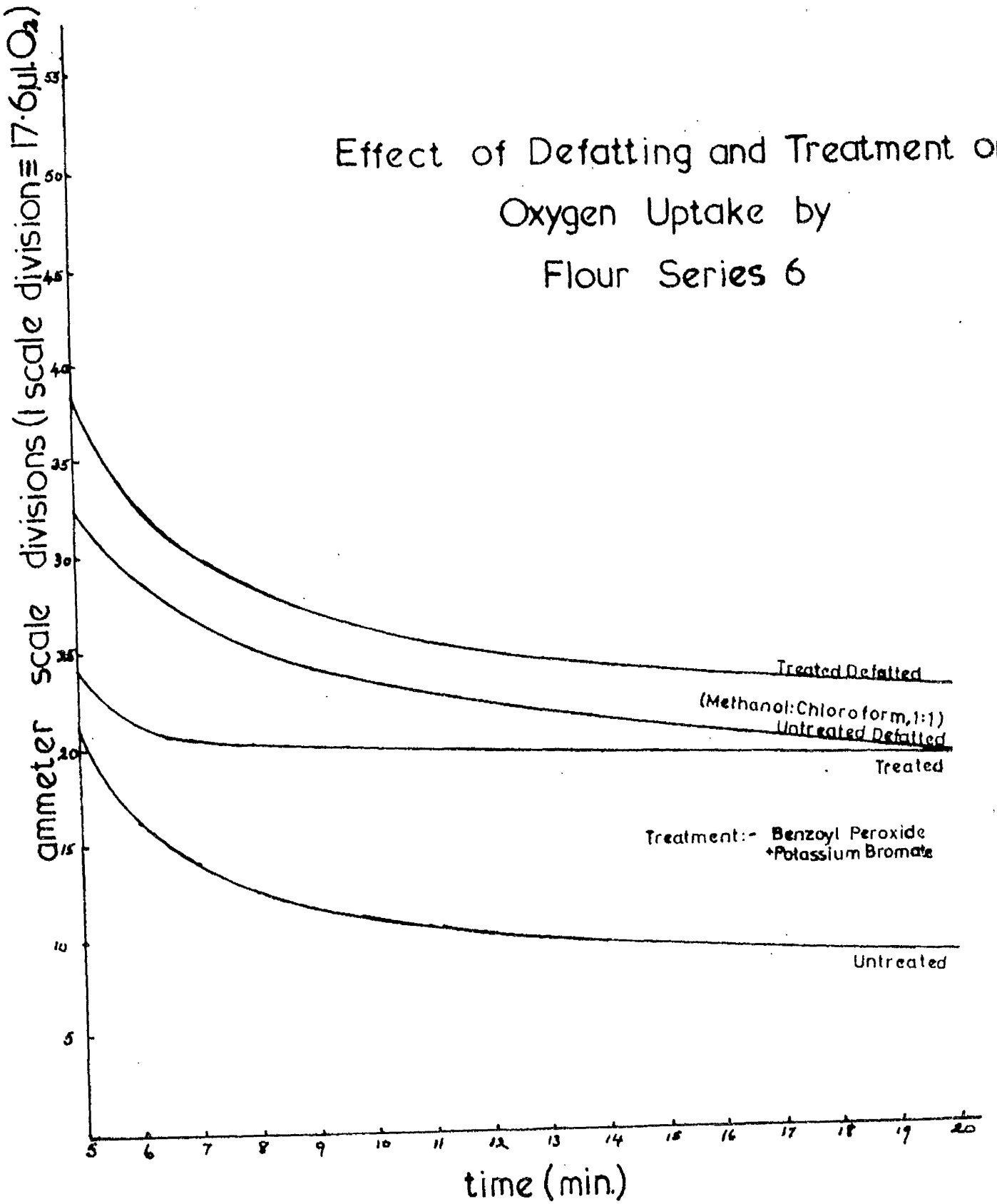

Effect of Treatment on
Oxygen Uptake by
Flour Series 2
Defatted
ammeter scale divisions (1 scale division ≡ 17·6 µl. O_2)
time (min.)
Treated Defatted
(Petroleum Ether 40°-60°)
Treatment:- Benzoyl Peroxide
+ Potassium Bromate
Treated Control
Untreated Defatted
(Petroleum Ether 40°-60°)

Diagram 8


Effect of Defatting and Treatment on
Oxygen Uptake by
Flour Series 6
ammeter scale divisions (1 scale division ≡ 17·6µl O2)
55
50
45
40
35
30
25
20
15
10
5
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
time (min.)
Treated Defatted
(Methanol: Chloroform, 1:1)
Untreated Defatted
Treated
Treatment:- Benzoyl Peroxide
+Potassium Bromate
Untreated

PART 4

The Effect of Adding Lipid on the Oxygen Uptake by Defatted Flour Suspensions

A. Samples Studied

An untreated Spring flour was used to determine the effect of the addition of lipid to defatted flour. The sample was:-

Spring Flour:- Series 7 Untreated

The flour was extracted with methanol:chloroform (1/1,v./v.) as described under PART 3B, No. 2.

B. Procedure for Extraction and Redeposition of Flour Lipid

Flour (100g.) was slurried with 200 ml. methanol in a glass jar. 300 ml. of methanol:chloroform (1/1,v./v.) were added and the suspension thoroughly stirred for 30 min. The glass jar was fitted with a lid carrying a nitrogen inlet, and two wide glass tubes connected to water pumps. After removal of the bulk of the solvent the jar was connected to a rotary vacuum pump, and the final traces of solvent were removed. The flour was allowed to stand in a cold store (4° C.) for a few days to restore the moisture content to its former level.

C. Linoleic Acid

0.75 ml. pure linoleic acid (Hormel Institute) was dissolved in 5 ml. pure ethyl alcohol. 1 ml. portions of this solution (= 0.135g. linoleic acid, ρ = 0.9025g./cc.) were added to the supporting electrolyte immediately before the commencement of each experiment. This addition corresponded to 45 mg./6g. of defatted flour.

D. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p.52 and p.60 respectively.

E. Results

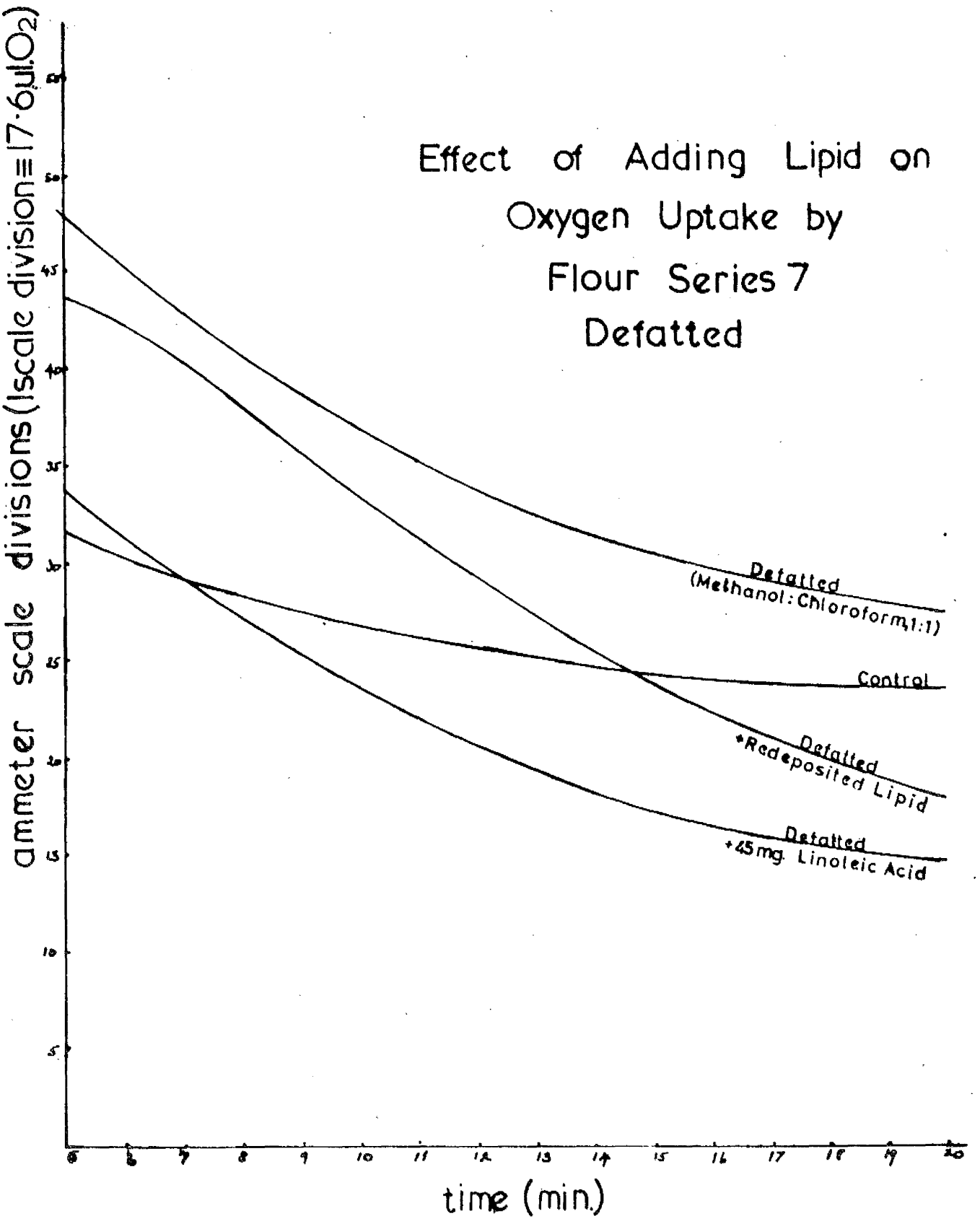
The results are reported in TABLE 14, and are illustrated by the graph Diagram 9 p.92.

TABLE 14

Effect of Adding Lipid to Defatted Flour Suspensions

Flour Series 7	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	372	143	85.8	56
Untreated Defatted	85	361	74.3	48
Defatted + Redeposited Lipid	154	461	103	66
Defatted + Linoleic Acid	337	335	112	72

Diagram 9


Effect of Adding Lipid on
Oxygen Uptake by
Flour Series 7
Defatted
ammeter scale divisions (1scale division ≡ 17·6µl.O2)
time (min.)
Defatted
(Methanol : Chloroform, 1:1)
Control
Defatted
*Redeposited Lipid
Defatted
+45mg. Linoleic Acid

PART 5

The Effect of Antioxidant on the Oxygen Uptake by Flour Suspensions

A. Samples Studied

A series of three Spring flours were used to determine if the presence of an antioxidant could influence the oxygen uptake process. The flours were:-

Spring Flour:- Series 3 Untreated

Series 3 Untreated, defatted with petroleum ether (Boiling range 40° - 60°C.)

Spring Flour:- Series 7

Spring Flour:- Series 8

B. Nordihydroguaiaretic Acid (NDGA)

For Flour Series 3 and 8:- 0.0667g. nordihydroguaiaretic acid was dissolved in 2 ml. ethyl alcohol. The solution was added to 150 ml. of flour suspension immediately after the completion of mixing. The concentration of the antioxidant was, therefore, 66.7 mg./6g. of flour.

For Flour Series 7:- 0.027g. nordihydroguaiaretic acid was dissolved in 2 ml. ethyl alcohol. The solution was added to 450 ml. of the supporting electrolyte (0.125 M.KCl) prior to the preparation of the flour suspension. The concentration of the antioxidant was, therefore, 9.0 mg./6g. of flour.

C. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p.52 and p.60 respectively.

D. Results

The results are reported in TABLES 15 - 17. The effect of the antioxidant on the oxygen uptake of Flour Series 8 is illustrated by the graph Diagram 10 p. 97.

TABLE 15.

Effect of Antioxidant on the Oxygen Uptake of Flour Suspensions

Flour Series 3	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated Defatted	314	428	124	80
Untreated Defatted + 66.7 mg. NDGA	411	382	132	86

TABLE 16.

Effect of Antioxidant on the Oxygen Uptake of Flour Suspensions

Flour Series 7	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis	Available Oxygen Absorbed% 0-20 min.
Untreated	372	143	85.8	56
Untreated + 9.0 mg. NDGA	494	107	100	65

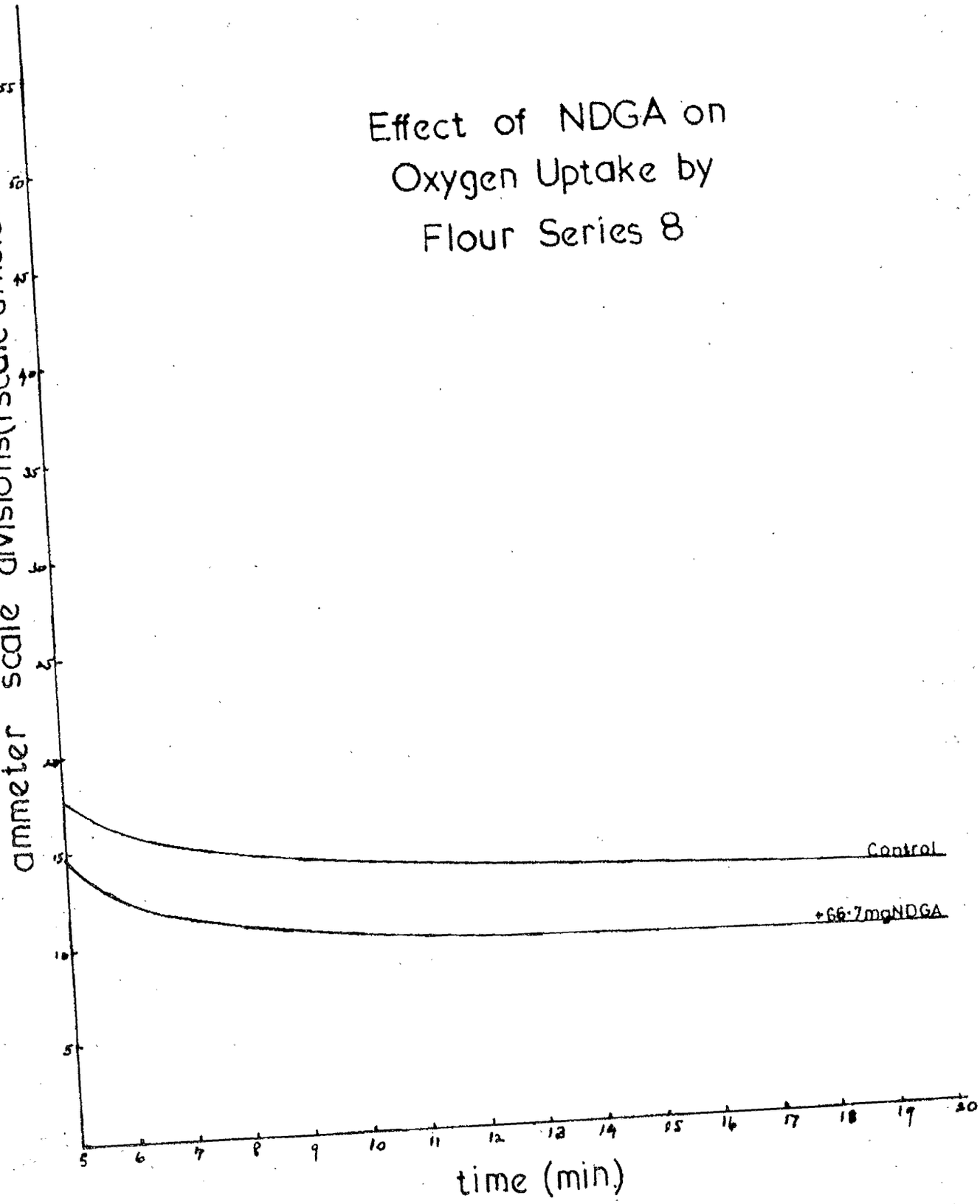
TABLE 17.

Effect of Antioxidant on the Oxygen Uptake of Flour Suspensions

Flour Series 8	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis	Available Oxygen Absorbed% 0-20 min.
Untreated	615	90	118	76
Untreated $\pm$ 66.7 mg. NDGA	670	95	128	83

Diagram 10

Effect of NDGA on Oxygen Uptake by Flour Series 8



PART 6

The Effect of Sulphydryl Blocking Agents and Reduced Glutathione on the Oxygen Uptake by Flour Suspensions

A. Samples Studied

A series of three Spring flours were used to determine the effect of sulphydryl blocking agents, and reduced glutathione, on the oxygen uptake by flour suspensions. The flours were:-

Spring Flour:- Series 3 Untreated

Series 3 Untreated, defatted with petroleum ether (Boiling range 40 - 60° C.)

Spring Flour:- Series 5 Untreated

Spring Flour:- Series 7 Untreated.

B. Sulphydryl Blocking Agents

(1) Iodoacetic Acid

A solution containing 5 mg./ml. iodoacetic acid was used for these experiments. Amounts corresponding to 20 mg., 25 mg., and 50 mg. iodoacetic acid were added to the supporting electrolyte before the latter was diluted to a final volume of 450 ml. These additions corresponded to 6.7 mg., 8.3 mg., and 16.7 mg. iodoacetic acid per 6g. of flour, or about 4.6, 5.75, and 11.5 times the total sulphydryl content of the flour.

(2) p-Chloromercuribenzoic Acid (sodium salt)

A solution containing 10 mg./ml. sodium p-chloromercuribenzoate (PCMB) was used for these experiments. Amounts corresponding to

10 mg., and 30 mg. PCMB were added to the supporting electrolyte before the latter was diluted to a final volume of 450 ml. These additions corresponded to 3.3 mg., and 10 mg. sodium p-chloromercuribenzoate per 6g. of flour, or about 1.1 and 3.3 times the total sulphydryl content of the flour.

(3) Iodoacetamide

A solution containing 8 mg./ml. iodoacetamide was used for these experiments. Amounts corresponding to 8 mg., and 24 mg. iodoacetamide were added to the supporting electrolyte before the latter was diluted to a final volume of 450 ml. These additions corresponded to 2.7 mg., and 8 mg. iodoacetamide per 6g. of flour, or about 1.85 and 5.5 times the total sulphydryl content of the flour.

The amounts of the above reagents are similar to those employed by Mecham (116) and were calculated on an estimated flour sulphydryl content of 1.3 µeq./g. (123,124).

C. Reduced Glutathione

A small amount of finely powdered reduced glutathione, equivalent to 100 µeq. of sulphydryl groups (0.0307g.), was mixed with the flour immediately prior to the commencement of the experiment. This addition corresponded to 33.3 µeq. of sulphydryl groups per 6g. of flour.

D. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p. 52 and p. 60 respectively.

E. Results

The results are reported in TABLES 18 - 20. The effects of iodoacetamide on Flour Series 5, and reduced glutathione on Flour Series 7, are illustrated on p. 103 and p. 104, Diagrams 11 and 12, respectively.

TABLE 18.

Effect of Sulphydryl Blocking Agents on the Oxygen Uptake of Flour Suspensions

Flour Series 3	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.
Untreated Defatted	314	428	124	80
Untreated Defatted + 6.7 mg. Iodoacetic Acid	543	188	122	79
Untreated Defatted + 8.3 mg. Iodoacetic Acid	416	240	109	71
Untreated Defatted + 16.7 mg. Iodoacetic Acid	434	220	109	71
Untreated Defatted + 3.3 mg. PCMB	411	326	123	80
Untreated Defatted + 10 mg. PCMB	649	99	125	81

TABLE 19.

Effect of Sulphydryl Blocking Agents on the Oxygen Uptake of Flour Suspensions

Flour Series 5	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	507	242	125	81
Untreated + 2.7 mg. Iodoacetamide	615	155	128	83
Untreated + 8 mg. Iodoacetamide	698	88	131	85

TABLE 20.

Effect of Reduced Glutathione on the Oxygen Uptake of Flour Suspensions

Flour Series 7	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. 1g. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.
Untreated	372	143	85.8	56
Untreated + 10.2 mg. Reduced Glutathione	508	217	121	78

Diagram II

Effect of Iodoacetamide on Oxygen Uptake by Flour Series 5

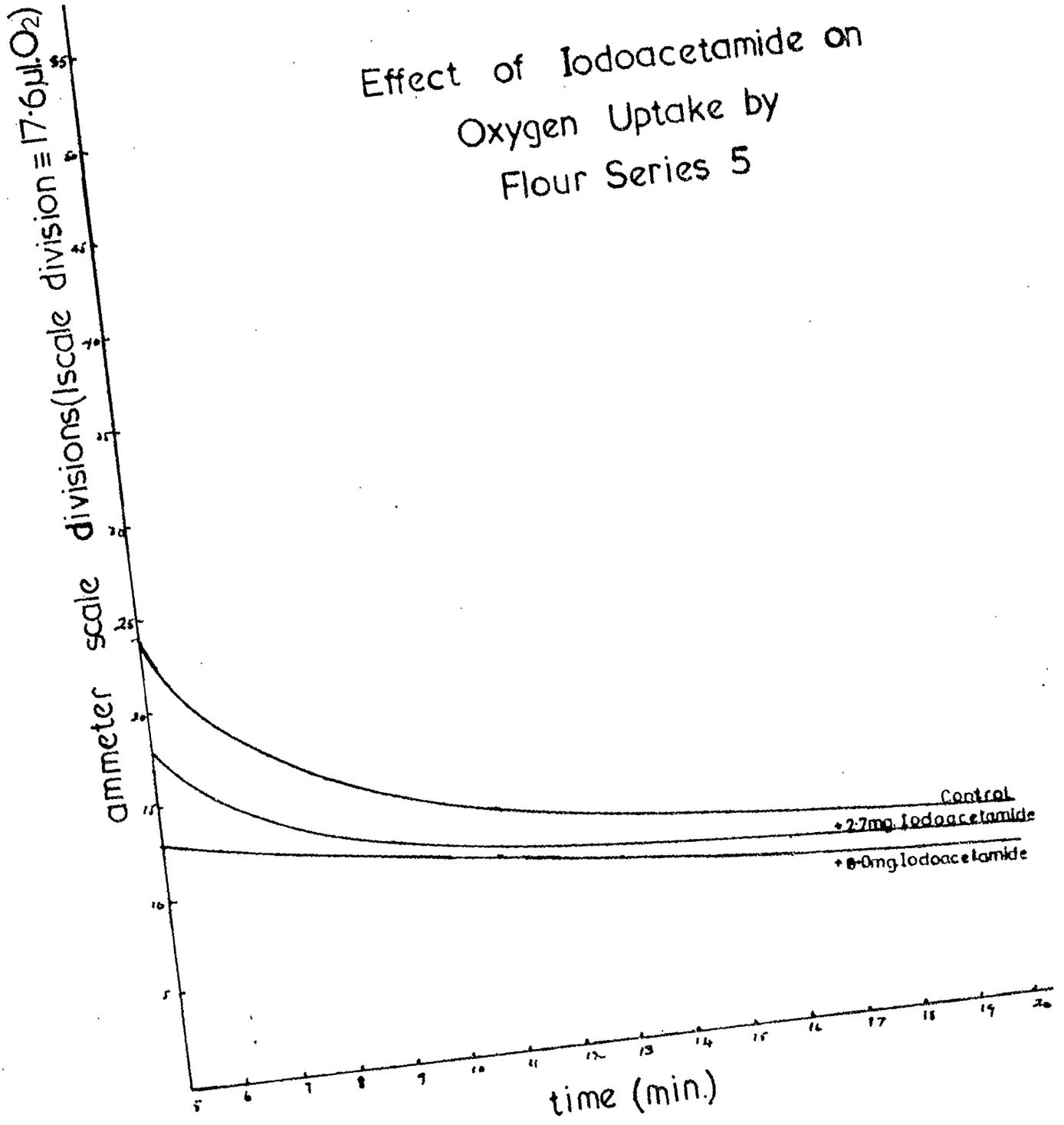
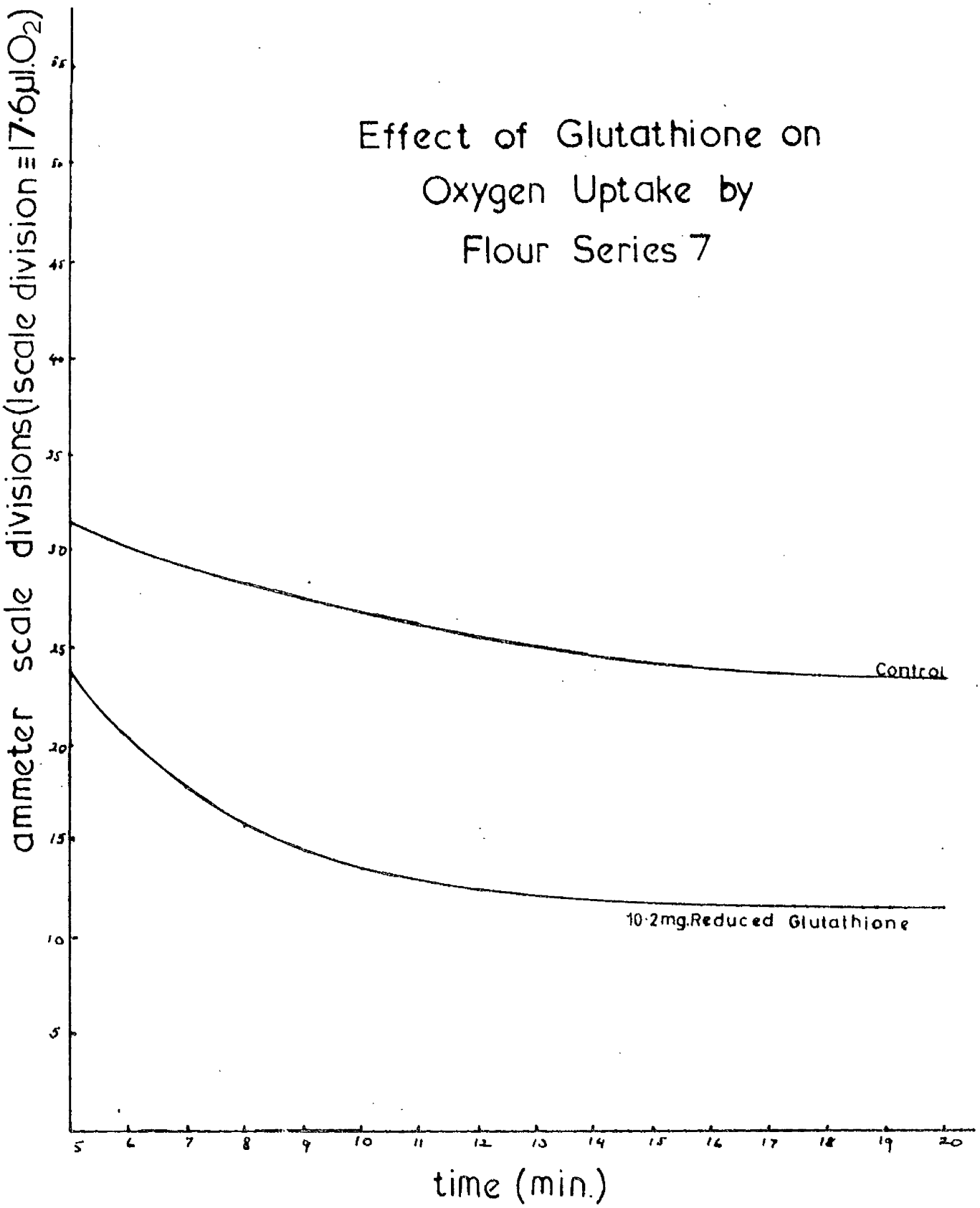


Diagram 12


Effect of Glutathione on
Oxygen Uptake by
Flour Series 7
ammeter scale divisions (1 scale division ≡ 17·6 µl. O_2)
55
50
45
40
35
30
25
20
15
10
5
Control
10·2 mg. Reduced Glutathione
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
time (min.)

PART 7

The Effect of Protein Content on the Oxygen Uptake by Flour Suspensions

A. Samples Studied

A series of two Spring flours were used for these experiments. These flours had been subjected to impact milling, followed by air classification into high and low protein fractions. The samples were given the following designations:-

Spring Flour:- Series 9 - Before impact milling.
Series 9 - After impact milling.
Series 9 - High protein fraction.
Series 9 - Low protein fraction.
Spring Flour:- Series 10 - High protein fraction.
Series 10 - Low protein fraction.

B. Protein Estimation

The protein nitrogen of the flour was estimated by the macro-Kjeldahl method. The catalyst was a copper sulphate-selenium mixture (3 parts powdered $CuSO_4$: 1 part powdered Se). The result was converted to % protein by using the factor 5.7 (166).

C. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p. 52 and p. 60 respectively.

D. Results

The results are reported in TABLES 21 - 22. The effect of the protein 'shift' on oxygen uptake is illustrated on p.107 Diagram 13.

TABLE 21.

Effect of Protein Content on the Oxygen Uptake by Flour Suspensions

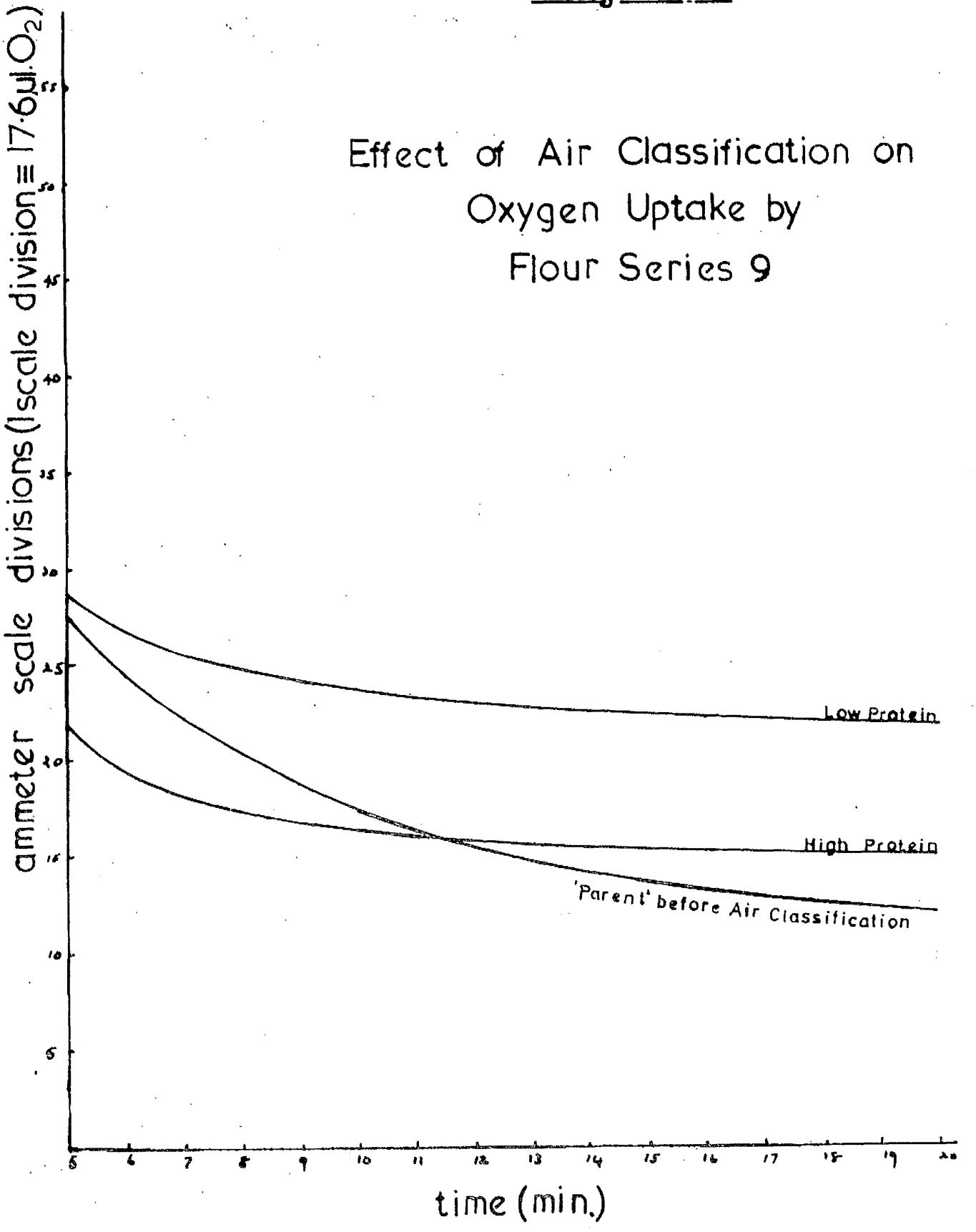
Flour Series 9	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.	Protein Content (%wet basis)
Flour to Kek Mill	441	264	118	76	-
Flour from Kek Mill	440	272	119	77	-
High Protein Fraction	543	120	110	72	13.7
Low Protein Fraction	423	120	90.5	59	6.8

TABLE 22.

Effect of Protein Content on the Oxygen Uptake by Flour Suspensions

Flour Series 10	Oxygen Uptake 0-5 min. ($\mu l.O_2$.)	Oxygen Uptake 5-20 min. ($\mu l.O_2$.)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2$.)	Available Oxygen Absorbed% 0-20 min.	Protein Content (% wet basis)
High Protein Fraction	416	217	105	68	15.7
Low Protein Fraction	284	257	90.2	58	6.4

Diagram 13


Effect of Air Classification on
Oxygen Uptake by
Flour Series 9
ammeter scale divisions (1 scale division ≡ 17·6 µl. O2)
time (min.)
Low Protein
High Protein
'Parent' before Air Classification

PART 8

The Influence of pH on the Oxygen Uptake by Flour Suspensions

A. Samples Studied

A series of two Spring flours were used in these experiments, which were originally intended to demonstrate the influence of cyanide on the oxygen uptake process. The observed effect with cyanide was found to be due to a change in the pH of the flour suspension. The samples were given the following designations:-

Spring Flour:- Series 3 Untreated

Series 4 Untreated

B. Potassium Cyanide

Sufficient potassium cyanide ('Analar') was added to the supporting electrolyte (450 ml. 0.125 M.KCl) to make the final concentration equivalent to 1×10^{-4} M.KCN, 5×10^{-4} M.KCN, 7.5×10^{-4} M.KCN and 1×10^{-3} M.KCN. The flour suspension was prepared in the usual manner, the pH of the suspension being recorded on a pH meter (Cambridge Instrument Co.).

C. Potassium Hydroxide

1.25 ml. of 2% potassium hydroxide ('Analar') was added to the supporting electrolyte (450 ml. 0.125 M.KCl). The flour suspension was prepared in the usual manner, the pH of the suspension being recorded on the pH meter.

D. Cytochrome 'c' and Flour Solubles

2 ml. increments of a concentrated solution of cytochrome 'c' (exact strength unknown) were added to a flour suspension containing 1×10^{-3} M. KCN.

An extract of flour solubles was prepared so that 2 ml. was equivalent to the solubles from 0.65g. flour, 2 ml. increments were added to a flour suspension containing 1×10^{-3} M.KCN.

E. Apparatus and Method

See PART 2 and PART 3 of the EXPERIMENTAL section of this thesis, p.52 and p.60 respectively.

F. Results

The results are reported in TABLES 23 - 24. The influence of pH on the oxygen uptake of Flour Series 4 is illustrated on p.111 Diagram 14.

The addition of a total of 8 ml. concentrated cytochrome 'c' and 10 ml. flour solubles, did not bring about any change in the oxygen uptake of the suspension containing 1×10^{-3} M. KCN.

TABLE 23.

Influence of pH on the Oxygen Uptake by Flour Suspensions

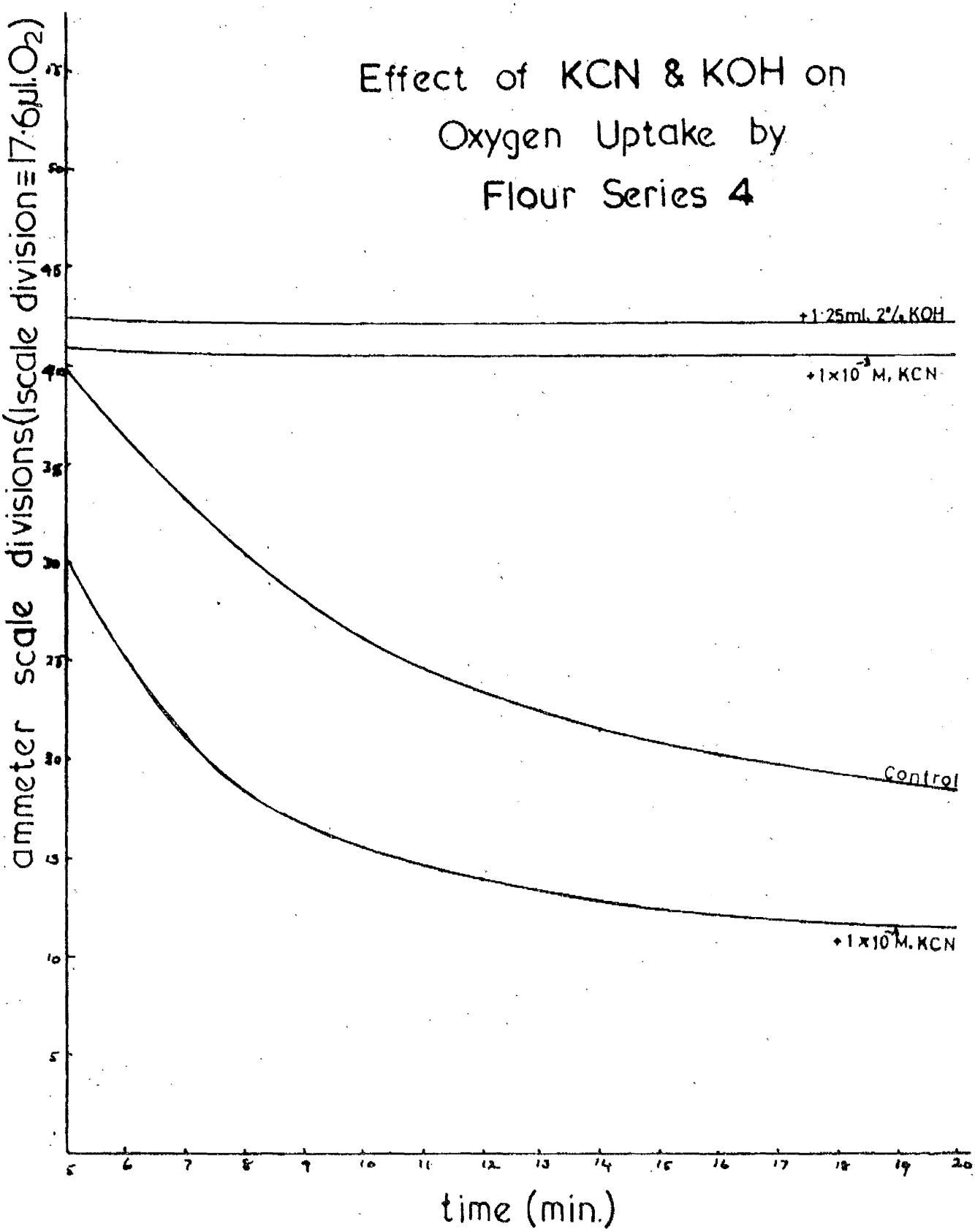
Flour Series 3	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min,	pH of Suspension
Untreated	418	354	129	83	6.12
Untreated $1 \times \overset{+}{1}0^{-4}$ M.KCN	425	305	122	79	-
Untreated $5 \times \overset{+}{1}0^{-4}$ M.KCN	302	358	110	71	-
Untreated $7.5 \overset{+}{\times} 10^{-4}$ M.KCN	293	328	103	67	-
Untreated $1 \times \overset{+}{1}0^{-3}$ M.KCN	328	23	58.5	38	7.88

TABLE 24.

Influence of pH on the Oxygen Uptake by Flour Suspensions

Flour Series 4	Oxygen Uptake 0-5 min. ($\mu l.O_2.$)	Oxygen Uptake 5-20 min. ($\mu l.O_2.$)	Oxygen Uptake 0-20 min. lg. Basis ($\mu l.O_2.$)	Available Oxygen Absorbed% 0-20 min.	pH of Suspension
Untreated	227	372	100	65	6.13
Untreated $1 \times \overset{+}{1}0^{-4}$ M.KCN	399	325	121	78	6.38
Untreated $1 \times \overset{+}{1}0^{-3}$ M.KCN	205	6	35	23	7.60
Untreated $1.25 \overset{+}{}$ ml.2% KOH	180	5	31	20	7.16

Diagram 14


Effect of KCN & KOH on
Oxygen Uptake by
Flour Series 4
ammeter scale divisions (1 scale division = 17·6 µl. O_2)
time (min.)
+1·25ml. 2% KOH
+1×10⁻³ M. KCN
Control
+1×10⁻⁴ M. KCN

D I S C U S S I O N

DISCUSSION

General Observations on the Oxygen Uptake by Flour Suspensions

In the following discussion the results are presented in the form of mean uptake values in a number of instances. By this means, the significance of certain features may be more clearly demonstrated. It should be noted that the individual results, which compose the mean values, may be obtained by reference to the appropriate PART of the RESULTS section. In the case of TABLES K,L,M the results have been expressed in the form of positive and negative deviations from a control experiment.

The procedure chosen for this study permitted the measurement of two parameters, firstly, the magnitude of oxygen absorption, and secondly, the time interval over which oxygen absorption takes place. The mean uptake values for the nine Spring flours studied are given in TABLE A.

TABLE A*

Uptake of Dissolved Oxygen by High Grade Spring Flour

Flour	Uptake 0-5 min. ($\mu l.O_2.$)	Uptake 5-20 min. ($\mu l.O_2.$)	Total Uptake ($\mu l.O_2.$)
Flour Series 1-9	470	240	710

*Results expressed on a 6g. basis in TABLES A-Q.

From the above values it is seen that flour takes up oxygen very rapidly when wetted. The uptake curve (Diagram 1.p.75) may be divided into an initial period (0-5 min.) of rapid oxygen absorption, followed by a secondary period (5-20 min.) in which the rate of oxygen absorption declines. The initial period accounts for about two-thirds of the total oxygen taken up by the flour, and after 20 min. the amount of oxygen being removed from the solution is extremely small.

The results may be compared with the findings of previous workers (TABLE B), who used manometric devices for the study of oxygen absorption from flour batters (10) and flour doughs (11).

TABLE B

Comparison of Oxygen Uptake Results with those of Other Workers

Author	Form of Apparatus	Flour Studied	Form of Wetted Flour	Oxygen Supply	General Order of Results
Cosgrove (10)	Manometric	Manitoba	Batter	O_2 from Air	600-1000 µl.O_2./25g./20 min.
Smith and Andrews (11)	Manometric	Patent	Dough	O_2 Atmosphere	$\simeq$ 8000 µl.O_2./50g./20 min.
Cross	Polarographic	High Grade Spring	Suspension	Dissolved O_2	515-835 µl.O_2./6g./20 min.

It will be seen from an examination of TABLE B that the results are not strictly comparable, owing to the different conditions used by each worker. The manometric methods employed had two main disadvantages:-

(1) There was a general lack of sensitivity in recording small changes of oxygen pressure. As pointed out by Hawthorn (135), the data of Cosgrove, and Smith and Andrews (10,11,) would suggest that their equipment would not measure changes in oxygen concentration below about 5 ml. per lb. of flour (200-300 $\mu l.O_2./25g.$), with much certainty.

(2) The response of the manometer depended on the rate of equilibrium of oxygen between the gas phase and the dough or batter. Oxygen must diffuse into the liquid surrounding the flour particles before being absorbed, consequently the response depended on the diffusion gradients existing in the system.

The method reported in this thesis measures the rapid removal of dissolved oxygen from flour suspensions with high sensitivity. As reported in the experimental section (p.62), the presence of air above the suspension has an inappreciable effect on the amount of oxygen taken up during the experimental period. This is probably due to oxygen being removed from the suspension at a much faster rate than it can be supplied by diffusion.

The results of Cosgrove, which correspond to 140-240 $\mu l.O_2./6g./20$ min., depend on the diffusion of air into a

flour batter. The rate of stirring, air/batter surface ratio, and type of flour studied will all have an important bearing on the results. It should be mentioned that Cosgrove removed any traces of carbon dioxide evolved during the experiment by absorption. This feature was omitted by Smith and Andrews in their apparatus. Further, Cosgrove measured the oxygen absorption at 25° ± 0.05° C. by immersing the apparatus in a thermostat tank; Smith and Andrews reported that their measurements were made at a laboratory temperature of 'about 25° C.'

Smith and Andrews have shown that the mixing of a flour dough is accompanied by a large uptake of oxygen. It would seem probable that the proximity of the flour particles would cause variations in the oxidation pathways of doughs, batters, and suspensions. The results for a patent flour dough mixed under oxygen are, however, of the same order of magnitude as those reported in this thesis (TABLE C).

TABLE C

Uptake of Oxygen from an Oxygen Atmosphere by a Patent Flour - Data of Smith and Andrews (a,b), (11).

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)
Patent Flour	480	480	960

(a) The results have been re-calculated on a 6g. basis. The original figures were 4 ml. per 50g. after 5 min., 8 ml. per 50g. after 20 min.

(b) After 20 min. no further absorption was observed.

Both Smith and Andrews, and Cosgrove, considered that the oxygen absorption was due to oxidation of linoleic and linolenic acids, under the influence of the enzyme lipoxidase. The addition of linoleic acid to defatted flour brought about an increase in oxygen absorption, equivalent to a mole-for-mole oxidation.

Morrison (12) has pointed out that the linoleic and linolenic acid content of flour could not account for the magnitude of the results observed by Smith and Andrews. This author has demonstrated that other free fatty acids are oxidised during dough mixing, probably by a general mechanism such as β- oxidation. The amount of oxygen required for such an oxidation, together with that

required for concurrent lipoxidase oxidation, is of the order noted by Smith and Andrews in their experiments.

Although lipid oxidation is undoubtedly involved in the oxygen uptake process, there are two other systems which may have an additional influence on the amount of oxygen removed from solution. These systems involve the sulphydryl groups of flour proteins, which may undergo direct oxidation (65,119,133), or oxidation as a result of prior peroxidation of flour lipid (133). These two mechanisms are believed to compete for oxygen (137).

The experiments, which are discussed in the following sections, were designed to determine the inter-relationship of various flour components on the oxygen absorption process. The polarographic determination of oxygen can provide an overall picture of the reactions occurring in the flour suspension. In the discussion, the results will be related to the findings of other workers who have studied specialised aspects of wheat flour oxidation.

Effect of Treatment on the Oxygen Uptake of Flour Suspensions

Treatment of Spring flours with oxidising agents has a marked effect on the oxygen uptake curve. It is seen from TABLE D that benzoyl peroxide, chlorine dioxide and potassium bromate, all significantly reduce the amount of oxygen taken up by the flour.

TABLE D

Effect of Treatment on Oxygen Uptake of Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)
Flour Series 1 untreated	620	215	835
Benzoyl Peroxide treated	585	92	677
Chlorine Dioxide treated	596	88	684
Potassium Bromate treated	554	84	638
Fully treated	583	88	671

Although benzoyl peroxide is added for its bleaching effect, potassium bromate for its improving effect and chlorine dioxide for its combined bleaching and improving action, there is no significant difference in their effect of reducing the oxygen uptake. This would indicate that the oxygen uptake mechanism is sensitive to both classes of oxidising agents in a non-specific manner. The effect of the treatments does not appear to be cumulative i.e. the fully treated flour shows similar uptake values to the flour subjected to individual treatments. It would appear, therefore, that there is a limit to the extent to which the uptake may be reduced by using these oxidising agents.

Further examples, indicating the effect of oxidising agents in reducing the oxygen uptake, are to be found in a study of Flour Series 2-7. The mean uptake values for untreated and treated flours are given in TABLE E.

TABLE E

Effect of Treatment on Oxygen Uptake of Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2.$)	Uptake 5-20 min. ($\mu l.O_2.$)	Total Uptake ($\mu l.O_2.$)
Flour Series 2-7 untreated	425	266	691
Flour Series 2-7 treated	389	151	540

In the case of Flour Series 2-7 the treatment employed was a combination of potassium bromate and benzoyl peroxide. It is interesting to note that treatment causes an overall reduction of about 170 $\mu l.O_2.$ with Flour Series 1, and an overall reduction of about 150 $\mu l.O_2.$ with Flour Series 2-7. These figures represent a 20% and 22% reduction of oxygen uptake respectively, when compared to the values of the untreated controls.

Treated flour absorbs about 35 $\mu l.O_2./6g.$ less than untreated flour during the initial period (0-5 min.), after wetting. In the second phase of uptake (5-20 min.), the treatment brings about a most marked reduction, treated flour taking up about 115 $\mu l.O_2./6g.$ less than the untreated flour.

Flour is treated with potassium bromate because it has a strengthening effect on the gluten proteins; chlorine dioxide has a

similar effect, although it also brings about a bleaching action (167). The exact nature of the improvement mechanism is still obscure, and only the reaction of potassium bromate with flour proteins has been studied in detail.

The effect of potassium bromate as a dough improver is considered to be related to its ability to oxidise sulphydryl groups (75,76,79). It has also been shown that oxygen and bromate compete for sulphydryl groups in flour dough (75,80,112).

A slow loss of sulphydryl groups has been observed in flour suspensions (119). The loss amounted to 10% in a 20 min. period. If the loss is considered to occur by simple aerobic oxidation (65,119,133), the oxygen required would be of the order of 5 µl./6g. calculated on a 1 µeq. sulphydryl = 1 µeq.O_2. basis.

The differences in uptake between treated and untreated flours could be partially accounted for by complete sulphydryl oxidation. This would account for a reduction of about 50 µl.O_2./6g. in the case of the treated flours. The reduction in uptake of 150 µl.O_2./6g. brought about by a variety of oxidising agents would indicate that these oxidising agents are acting on other flour constituents besides sulphydryl groups.

Similar effects, noted with benzoyl peroxide and chlorine dioxide, as well as potassium bromate, suggested that the oxidation of flour lipids may be involved. The implication of the linoleic acid-lipoxidase system in the oxygen uptake process is well

established (10,11,137). The effect of chlorine dioxide, benzoyl peroxide, and potassium bromate on the linoleic acid content of flour has been studied by various workers (168,169). No direct loss of linoleic acid has been demonstrated, although recent work (12,165,170) would indicate that potassium iodate and chlorine dioxide treatment can affect the overall pattern of free fatty acid oxidation in flour doughs. The mechanism is still obscure.

Sulphydryl groups may be oxidised in a coupled reaction involving the linoleic acid-lipoxidase system (131,133). Tsen and Hlynka (137) have demonstrated an increase in lipid peroxidation in doughs containing improvers, compared with untreated control doughs. It is considered that oxidation of sulphydryl groups by the improvers prevents these groups competing for oxygen with the lipoxidase system. It is, therefore, unlikely that removal of sulphydryl groups would cause a decrease in the amount of oxygen utilised by the lipoxidase system.

To sum up, the following points may be made:-

(1) Flour treated with both improving and bleaching agents takes up less dissolved oxygen than an untreated control.

(2) The reduction in uptake becomes more apparent in the second phase of the uptake process (5-20 min. after wetting).

(3) In the case of improving agents, it is considered that complete oxidation of sulphydryl groups could not account for the magnitude of the observed effects.

Effect of Removing Flour Lipid on the Oxygen Uptake of Flour Suspensions

(1) Defatted Untreated Flour

Defatting untreated Spring flour brought about a reduction in the amount of oxygen taken up from solution.

TABLE F

Effect of Defatting on Oxygen Uptake of Flour Suspensions [a]

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)
Control Series 1-3	502	280	782
Defatted Series 1-3	286	444	730
Control Series 6-7	465	179	644
Defatted Series 6-7	221	296	517

(a) Flour Series 1-3 was defatted by petroleum ether (Boiling range 40-60° C.) in a Soxhlet apparatus. Flour Series 6-7 was defatted by methanol:chloroform (1/1, v./v.) by the method of Morrison (12,165).

A. Petroleum Ether Extraction

Petroleum ether removes 'free lipid' material which amounts to about 50-70% of the total lipid in flour (138,171). The free

fatty acid fraction is involved in both peroxidation during dough mixing (137), and β-oxidation in simple flour water mixtures (12).

The decrease in uptake observed on defatting (TABLE F) is not considered to be related to inactivation of an oxidising system, during the petroleum ether extraction. The addition of flour lipids to petroleum ether extracted flour has shown that the lipoxidase system is undamaged (165), and that certain rheological properties have not been destroyed (138).

The decrease of 215 $\mu l.O_2./6g.$ immediately after wetting, and an overall decrease of 50 $\mu l.O_2./6g.$ after 20 min. would indicate that lipid oxidation occurs as soon as flour is wetted. More evidence is offered on this point in a later section (p.136). The presence of lipid material would seem to have a retarding influence on the amount of oxygen taken up by other substances in the suspension. This is suggested by the increase in the amount of oxygen absorbed during the period 5-20 min. (165 $\mu l.O_2./6g.$).

The total amount of oxygen utilised by lipid oxidation is difficult to ascertain, due to the nature of the results. It is certainly equivalent to 50 $\mu l.O_2./6g.$ and possibly as much as 215 $\mu l.O_2./6g.$, however it is not possible to make a more precise estimate.

Smith and Andrews (11) observed that defatted flour doughs, when mixed under oxygen, have a greatly reduced uptake compared with dough from unextracted flours. The findings of these workers cannot

be compared directly with the results presented in this thesis, owing to different experimental conditions. It is clear that the significance of the lipid material, in determining the magnitude of the uptake, is different in both experimental systems (TABLE G).

TABLE G

Effect of Removing Flour Lipid on Oxygen Uptake

Workers	Flour	Solvent	Experimental Conditions	Reduction in Uptake on Defatting	
				0-5 min.(%)	0-20 min.(%)
Smith and Andrews (11)	Patent	Pentane	Oxygen atmosphere. Dough. Manometric measurement	67	75
Cross	Spring	Petroleum ether	Dissolved oxygen. Suspension. Polarographic measurement.	43	7
Cross	Spring	Methanol: Chloro form	Dissolved oxygen. Suspension. Polarographic measurement.	53	20

The implication of lipid material in the oxygen uptake of flour doughs is well established. There would appear to be two mechanisms involved. The first was proposed by Smith and Andrews (11), who considered that oxygen is taken up by the lipoxidase-catalysed oxidation of linoleic acid. Tsen and Hlynka (137) have found that the petroleum ether extractable lipids undergo rapid peroxidation during dough mixing in an oxygen atmosphere. The degree of peroxidation was increased by the addition of lipoxidase.

The second mechanism was proposed by Morrison (12), who has demonstrated that other free fatty acids, besides linoleic acid, disappear rapidly during the mixing of flour-water sponges. This worker proposed a general enzymic oxidation, such as α or β-oxidation, to account for the results, and pointed out that linoleic acid content of flour could not account for the magnitude of the uptake observed by Smith and Andrews (11).

The decrease of oxygen uptake, observed during the initial period after wetting, is considered to be due to the removal of oxidisable lipid material by petroleum ether. Lipid oxidation appears to occur for only a short period after wetting, and clearly other substances, besides lipid, are oxidised to account for the observed results.

Evidence to support a 'non lipid' uptake has come from the work of various authors (3,133,138), who have demonstrated that defatted flours respond to mixing in oxygen, as measured by the

rheological characteristics of dough, or by improvement in baking quality. In this respect the findings of Narayanan and Hlynka (138) are of especial importance. These authors found that the oxygen response of petroleum ether extracted flours was greater than that of unextracted flours, as measured by the rheological properties of the respective doughs. The conclusion was drawn that the lipids exerted a protective influence against the improving action of oxygen.

It has been shown that improvers depress the amount of oxygen taken up by flour during the second phase after wetting (5-20 min.). Defatting, however, accelerates the rate at which oxygen is taken up during this time, and it is suggested that the presence of lipid depresses the amount of oxygen taken up by substances involved in the improvement process.

To sum up the following points may be made:-

(1) Flour can take up oxygen in the absence of 'free lipid' material.

(2) Removal of 'free lipid' slightly depresses the amount of dissolved oxygen taken up by Spring flour, in the 20 min. experimental period.

(3) The effect of defatting is to reduce the amount of oxygen absorbed over the period 0-5 min. after wetting, and increase the amount absorbed over the period 5-20 min. after wetting.

(4) The amount of oxygen utilised by lipid oxidation is considered to be of the order 50-215 $\mu l.O_2./6g.$

B. Methanol : Chloroform Extraction

Methanol:chloroform (1/1, v./v.) is a more efficient defatting solvent than petroleum ether. Methanol:chloroform removes free lipid material as well as the more tightly bound phospho-and galacto-lipids of flour (12). This solvent mixture, however, brings about partial or complete enzyme inactivation in the defatted flour (165).

It is seen from TABLE G that methanol:chloroform extraction reduces the amount of oxygen taken up by the flour. The reduction is greater than that brought about by petroleum ether extraction. The overall reduction in uptake by petroleum ether extraction is 50 $\mu l.O_2./6g./20$ min., the overall reduction by methanol:chloroform extraction is 125 $\mu l.O_2./6g./20$ min.

Both petroleum ether and methanol:chloroform will remove 'free lipid' which is the only lipid fraction known to be concerned in oxidation in flour doughs (137), and flour-water sponges (12). The difference in uptake is tentatively considered to be due to enzymic inactivation brought about by the methanol:chloroform mixture. This enzymic system would be concerned with the oxidation of 'non-lipid' material.

Methanol:chloroform extracted flours can still absorb 80% of the oxygen taken up by a control flour over a 20 min. period. This is

strong evidence that a 'non-lipid' oxidation mechanism is involved in the uptake process. The pattern of results, over the experimental period, indicates that removal of lipid alters the oxidation pathways existing in the suspension.

To sum up the following points may be made:-

(1) An enzymic 'non-lipid' oxidation system may exist in flour which is partially, or totally, inactivated by methanol:chloroform treatment.

(2) Methanol:chloroform extracted flours can absorb 80% of the oxygen taken up by a control flour. This confirms the finding, that flour can take up oxygen in the absence of 'free lipid' material, obtained with petroleum ether extracted flours.

(2) Defatted Treated Flour

The following results represent the mean oxygen uptake of treated Spring flours, and for the corresponding defatted flours.

TABLE H

Effect of Defatting on Oxygen Uptake of Treated Flour Suspensions [a]

Flour	Uptake 0-5 min. ($\mu l.O_2.$)	Uptake 5-20 min. ($\mu l.O_2.$)	Total Uptake ($\mu l.O_2.$)
Treated Control Series 1-3	428	128	556
Treated Defatted Series 1-3	145	160	305
Treated Control Series 6-7	421	110	531
Treated Defatted Series 6-7	177	264	441

(a) Flour Series 1-3 was defatted by petroleum ether (Boiling range 40-60° C.) in a Soxhlet apparatus. Flour Series 6-7 was defatted by methanol:chloroform (1/1, v./v.) by the method of Morrison (12,165).

It is seen from TABLE H, that treated control flours take up more oxygen than treated defatted flours. This would be expected in view of the results obtained previously (p.129 and p. 131).

Considering Flour Series 1-3, an estimate may be made of the amount of oxygen involved in lipid oxidation. In the case of untreated flours the amounts of oxygen involved could only be estimated within wide limits, 50-215 $\mu l.O_2./6g.$ flour. This was due to the increase in oxygen absorption during the second phase after wetting (5-20 min.), which interfered with the measurement. In the case

of treated flours this effect has been considerably diminished. It is seen that the decrease on defatting, over the period 0-20 min., is almost the same as the decrease over the period 0-5 min. (TABLE H). This indicates that lipid oxidation occurs very rapidly after wetting. The amount of oxygen involved in lipid oxidation is of the order 250-285 $\mu l.O_2./6g.$

The increase observed on defatting, during the second phase of uptake (5-20 min.), is not as great as that observed in the case of defatted control flours (TABLE F). This supports earlier findings (p.124) which have related the effect of oxidising treatment to a depression in the second phase of uptake. The increase of about 30 $\mu l.O_2./6g.$ in the second phase for Flour Series 1-3, compared with an increase of about 155 $\mu l.O_2./6g.$ for Flour Series 6-7, would indicate that treatment has a more pronounced effect on the former flours. This is confirmed in TABLE I in which the effect of treatment on the defatted flours are compared.

TABLE I

Effect of Treatment on the Oxygen Uptake on Defatted Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)
Untreated Defatted Series 1-3	286	444	730
Treated Defatted Series 1-3	145	160	305
Untreated Defatted Series 6-7	221	296	517
Treated Defatted Series 6-7	177	264	441

It is seen from TABLE I that untreated defatted flours take up more oxygen than that taken up by treated defatted flours. This finding parallels the effect of treatment on unextracted flours.

For Flour Series 1-3 it is found that the effect of treatment on the defatted flour is most marked, the overall uptake being reduced by 58%. The effect of treatment on the corresponding unextracted flours produced a decrease of 29%. Sullivan (63) has shown that bromate acts more effectively on defatted flours than on ordinary flours, and a correlation between these findings may exist.

For Flour series 6-7, the effect of treatment is similar in the presence or absence of lipid. The reduction in the second phase (5-20 min.) is small, indicating that these flours are not so

susceptible to oxidising agents as Flour Series 1-3.

To sum up the following points can be made:-

(1) The amount of oxygen involved in lipid oxidation is of the order 250-285 $\mu l.O_2./6g.$ This is considered to be a more accurate estimate than that given previously, 50-215 $\mu l.O_2./6g.$ Lipid oxidation occurs extremely rapidly after the flour has been wetted.

(2) Treated defatted flours take up less oxygen than treated control flours.

(3) Treated defatted flours take up less oxygen than untreated defatted flours.

Effect of Adding Lipid Material to Defatted Flour

The following results show the effect of defatting on an untreated Spring flour, followed by redepositing the extracted lipid on to the flour. A further experiment was carried out in which the unsaturated fatty acid, linoleic acid, was added to the defatted flour in place of the flour lipid.

TABLE J

Effect of Adding Lipid Material to Defatted Flour

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)
Control Flour	372	143	515
Flour defatted with MeOH—$CHCl_3$ (1/1, v./v.)	85	361	446
Defatted flour with redeposited lipid	154	461	615
Defatted flour with linoleic acid	337	335	672

The addition of flour lipid to the extracted flour increased the overall uptake by 170 $\mu l.O_2$. The oxidation pattern is, however, very different from that of the control flour. The main differences are:-

(1) The overall uptake of the flour containing the redeposited lipid is greater than that of the original control flour.

(2) The relative proportions of oxygen taken up during both phases, 0-5 min. and 5-20 min., have been altered.

The reason for this behaviour is difficult to interpret. The results of Smith and Andrews (11) indicate that the addition of lipid to extracted first and second clear flour doughs, resulted in a slight increase in oxygen absorption compared with control doughs. This was only true for short mixing times of 15 min., and in the case of a patent flour there was no detectable difference between a control dough, and a dough made from extracted flour containing added lipid.

A possible explanation is that the redeposited lipid may be more susceptible to oxidation as a thin layer on the surface of the flour particles, than it was before extraction from the flour.

The addition of linoleic acid to the defatted flour markedly increased the first phase of oxygen absorption by 250 $\mu l.O_2$., the second phase remaining unaffected. Thus, it seems probable that linoleic acid, which represents about 60% of the

free fatty acid content of high grade flour (165,170,172), undergoes rapid oxidation when wheat flour is wetted.

The level of linoleic acid added was equivalent to 45 mg./6g. flour, and it is clear that only a small proportion of the linoleic acid had been oxidised after 5 min. The amount of oxygen utilised by linoleic acid in the period 0-5 min. after wetting is equivalent to 3.2 mg. linoleic acid. The fact that more linoleic acid was not oxidised is probably due to the partial inhibition of the lipoxidase or β-oxidation systems, which has been noted on defatting flour with a methanol:chloroform mixture (12).

The results show parallels with the findings of Smith and Andrews (11), who observed that defatting reduced the amount of oxygen taken up by a flour dough, and that the addition of flour lipids, or linoleic acid, restored the uptake to its original level. The main difference between the results is that the lipid appears to have a greater significance in determining the uptake of oxygen by doughs, than it does in flour suspensions.

To sum up the following points may be made:-

(1) The addition of extracted lipid to a methanol:chloroform extracted flour increased the amount of oxygen absorbed by the flour. The addition of the lipid did not restore the uptake pattern to that of an unextracted flour, indicating that extraction had affected the lipid oxidation system.

(2) The addition of linoleic acid to the extracted flour increased the amount of oxygen absorbed over the period 0-5 min., but not over the period 5-20 min. after wetting.

Effect of an Antioxidant on the Uptake of Dissolved Oxygen by Flour Suspensions

The antioxidant nordihydroguaiaretic acid (NDGA) was added to flour suspensions, to determine if any inhibition of the uptake process could be detected. The results of this series of experiments will be found in TABLE K.

TABLE K

Effect of Nordihydroguaiaretic acid on the Uptake of Dissolved Oxygen by Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)
* Defatted Flour Series 3 + 66.7 mg.NDGA compared with control	+ 95	- 45	+ 50
Flour Series 7 + 9 mg.NDGA compared with control	+120	- 35	+ 85
Flour Series 8 + 66.7 mg.NDGA compared with control	+ 55	+ 5	+ 60

* Extracted with petroleum ether (Boiling range 40-60° C.)

The effect of a high level of NDGA on Flour Series 7 and 8 is to slightly increase the overall oxygen uptake (0-20 min.), compared with a control experiment (no NDGA added). A similar uptake

pattern was observed on the addition of NDGA to a petroleum ether extracted flour, Series 3.

NDGA is an inhibitor of the lipoxidase catalysed oxidation of linoleic acid (173), and of the peroxidation of lipids during dough mixing in oxygen (137). It has been shown that flour lipids undergo oxidation when flour is wetted, and a decrease in oxygen absorption would be expected in flour suspensions containing the antioxidant. As a decrease in the oxygen absorption was not observed with Flour Series 7 and 8, it is concluded that NDGA does not inhibit lipoxidase activity in flour suspensions. In this connection Mahon and Chapman (175) found that NDGA was inactivated by flour. These workers concluded that the antioxidant was destroyed or complexed by some component of the flour.

The addition of a high level of NDGA to a flour-water sponge increased the loss of linoleic and linolenic acids (12), perhaps due to an acceleration in the decomposition of linoleate hydroperoxide (136). The increase observed in the uptake of the unextracted flours suggests a correlation between these findings may exist. The increased uptake found with the extracted flour indicates, however, that the effect of NDGA is independent of lipid material.

It has been observed that antioxidants could act as improvers in flour doughs mixed in air (80). The abstraction of a hydrogen atom from a phenolic antioxidant involves rearrangement to a

semi-quinone structure (174). Narayanan and Hlynka (138) proposed that semi-quinones act as improvers by a reaction involving thiol RSH. It is probable that the effect of NDGA on the oxygen absorption by flour suspensions is also related to its ability to be oxidised; however, a definite reaction mechanism has not been proposed.

To sum up, it may be stated that high levels of NDGA slightly increase the amount of oxygen taken up by flour when wetted. The reason for this finding is unknown, but it would appear that NDGA does not inhibit the lipoxidase system in flour suspensions and has an effect which is independent of lipid material.

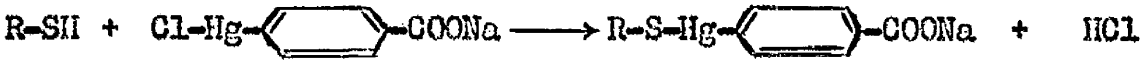
Effect of Sulphydryl Blocking Agents on the Uptake of Dissolved Oxygen by Flour Suspensions

Numerous authors (65,119,133) have found that the sulphydryl groups of flour proteins are susceptible to oxidation by atmospheric oxygen. Accordingly, the effects of specific sulphydryl blocking reagents upon the oxygen uptake of flour suspensions were investigated. The reagents employed to block the sulphydryl groups were iodoacetic acid, iodoacetamide, and para-chloromercuribenzoate (PCMB). The reactions of these reagents with sulphydryl groups are as follows:-

(i) Reaction of Iodoacetic Acid with Sulphydryl Groups

(ii) Reaction of Iodoacetamide with Sulphydryl Groups

(iii) Reaction of P-Chloromercuribenzoate with Sulphydryl Groups



The results in TABLE L indicate the differences in uptake between control flours and flours treated with the reagents.

TABLE L

Effect of Sulphydryl Blocking Agents on the Uptake of Dissolved Oxygen by Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2$.) Compared with control	Uptake 5-20 min. ($\mu l.O_2$.) Compared with control	Total Uptake ($\mu l.O_2$.) Compared with control
Series 5 + 2.7 mg. Iodoacetamide	+ 110	- 90	+ 20
Series 5 + 8 mg. Iodoacetamide	+ 190	- 155	+ 35
* Series 3 Defatted + 6.7 mg. Iodoacetic Acid	+ 230	- 240	- 10
Series 3 Defatted + 8.3 mg. Iodoacetic Acid	+ 100	- 190	- 90
Series 3 Defatted + 16.7 mg. Iodoacetic Acid	+ 120	- 210	- 90
Series 3 Defatted + 3.3 mg. PCMB	+ 100	- 100	0
Series 3 Defatted + 10 mg. PCMB	+ 335	- 330	+ 5

*) Extracted with petroleum ether (Boiling range 40-60° C.)

The amounts of the reagents used were in excess of those required to block the total sulphydryl groups in the flour sample, assuming a maximum sulphydryl content of 1.3 μeq./g. (123,124). Thus, the addition of 2.7 mg. and 8.0 mg. iodoacetamide was equivalent to

1.9 and 5.5 times the total sulphydryl content of the flour. For iodoacetic acid, the additions were equivalent to 4.6, 5.8, and 11.5 times the sulphydryl content, and for PCMB, 1.1 and 3.3 times the sulphydryl content. Similar amounts of sulphydryl reagents were employed by Mecham (116), to block sulphydryl groups during dough mixing.

The main effect of the sulphydryl reagents is to accelerate the rate of oxygen absorption, without greatly affecting the overall amount of oxygen taken up by the flour. This finding indicates that sulphydryl groups have an influence on the oxygen uptake process which is not related to their ability to undergo aerobic oxidation.

The results with the extracted and unextracted flours show a similar pattern, indicating that the acceleration effect is independent of lipid material. There is a slight increase in the amount of oxygen absorbed by the unextracted flour. This may be explained by an increase in the degree of peroxidation of flour lipids, which has been observed on addition of iodoacetic acid, PCMB, and N-ethylmaleimide to flour doughs (128,137). Dahle and Sullivan (128) state the increase in peroxides found in the presence of N-ethylmaleimide was due to the antioxidant effect of the -SH groups on the lipid oxidation.

The defatted flour shows a slight depression of uptake with iodoacetic acid, but PCMB has no effect in this respect. The reason for this difference is not understood, but it may be related to the different affinities of these reagents for sulphydryl groups (173).

The blocking of sulphydryl groups allows a 'non-lipid' oxidation to proceed at a faster rate without greatly affecting the overall uptake. Thus, there would appear to be two types of sulphydryl groups present in flour, i.e. those undergoing aerobic oxidation (65,119,133), and those which have a retarding influence on the rate of uptake.

Evidence to support the theory that there are two types of sulphydryl groups in flour was obtained by the addition of reduced glutathione (a tripeptide containing 1 eq. sulphydryl/mole) to a flour suspension.

TABLE M

Effect of Reduced Glutathione (GSH) on the Uptake of Dissolved Oxygen by Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2.$)	Uptake 5-20 min. ($\mu l.O_2.$)	Total Uptake ($\mu l.O_2.$)
Flour Series 7 + 10.2 mg. GSH compared with control	+ 135	+ 75	+ 210

The addition of reduced glutathione increased the total oxygen absorption by 210 $\mu l.O_2.$ (0-20 min.). This additional absorption is close to the theoretical value of 190 $\mu l.O_2.$ which represents complete oxidation of the glutathione. The autoxidation

of a similar quantity of reduced glutathione in aqueous suspension is much slower, the amount oxidised after 20 min. being too small to be measured accurately (less than 5% of the total) (47).

It would be expected, therefore, that the blocking of sulphydryl groups will lead to a small reduction in the oxygen uptake, as aerobic oxidation can no longer occur. To account for the acceleration effect it is postulated that there are certain sulphydryl groups in flour which have a retarding influence on the rate of oxygen absorption. It is well known that certain enzymes are dependent on sulphydryl groups for their activity. It is considered that an enzyme system is present in flour which normally depresses the rate of oxygen absorption; when this system is inactivated by sulphydryl reagents the uptake of oxygen proceeds at an increased rate.

Effect of Impact Milling and Air Classification on the Uptake of Dissolved Oxygen by Flour Suspensions

The oxygen uptake of various fractions of flour, each containing differing amounts of protein, was studied. The fractions had been obtained by the process of impact milling, followed by air classification.

In the impact milling process, size reduction of the flour particles is achieved in a pin-disc mill, with consequent separation of starch granules from their surrounding protein matrix. The flour is then subjected to a process of air classification which separates the flour into fine, medium and coarse particles depending on their density and projected area in an air stream. The fine fraction, which consists of particles less than 20μ in diameter, is made up of fragments of protein set free during impact milling, together with small detached starch granules. The proportion of protein to starch is much higher than in the original flour. The medium fraction consists of particles from 20-40μ in diameter, and contains a large proportion of free starch granules. This fraction has a relatively low protein content. Above 40μ the particles are mainly unfragmented 'chunks' of endosperm cells and contain protein at approximately the same level as the original flour.

The effect of impact milling on Flour Series 9 is shown in TABLE N.

TABLE N

Effect of Impact Milling on the Uptake of Dissolved Oxygen by Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2.$)	Uptake 5-20 min. ($\mu l.O_2.$)	Total Uptake ($ul.O_2.$)
Series 9 Before impact milling	441	264	705
Series 9 After impact milling	440	272	712

It is seen that the results before, and after, impact milling are similar. The oxygen absorption mechanism is, therefore, undamaged and the effective surface area of the particles is not an important factor in influencing the uptake of oxygen.

The effect of air classification on Flour Series 9 and 10 is shown in TABLE P.

TABLE P

Effect of Air Classification on Uptake of Dissolved Oxygen by Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2$.)	Uptake 5-20 min. ($\mu l.O_2$.)	Total Uptake ($\mu l.O_2$.)	Protein Content (%)
Series 9 High Protein	543	120	663	13.7
Series 9 After impact milling	440	272	712	-
Series 9 Low Protein	423	120	543	6.8
Series 10 High Protein	416	217	633	15.7
Series 10 Low Protein	284	257	541	6.4

The oxygen uptake of the high protein flour (Series 9), during the period 0-5 min. after wetting, is 100 $\mu l.O_2$. greater than that of the parent flour. The uptake of the low protein flour is about 20 $\mu l.O_2$. less than that of the parent flour during the same period. The high protein fraction, therefore, contains a greater proportion of material undergoing rapid oxidation compared with the low protein fraction.

It has been pointed out that the uptake during the period 0 - 5 min. is influenced by lipids. Air classification is known

to increase the lipid content of the high protein fraction (165,176). The difference in initial oxygen absorption may be attributed to a redistribution of lipid material between the two flour fractions.

Although the parent flour, Series 10, was not available, the difference in initial uptake (0-5 min.) between the high and low protein fractions was of a similar magnitude to that observed with the corresponding fractions of Flour Series 9.

Wrigley (177) has recently demonstrated similarities in the composition of protein from fine and medium fractions obtained after classification of flour in a Microplex air classifier. Further, Axford et al. (178) have shown that the protein in the different air classified flour fractions, did not show any variation with respect to sulphydryl and disulphide contents. In view of these findings, the differences in uptake observed between high and low protein fractions may be due to differences in gross protein content, but not to variations in the composition of the classified protein.

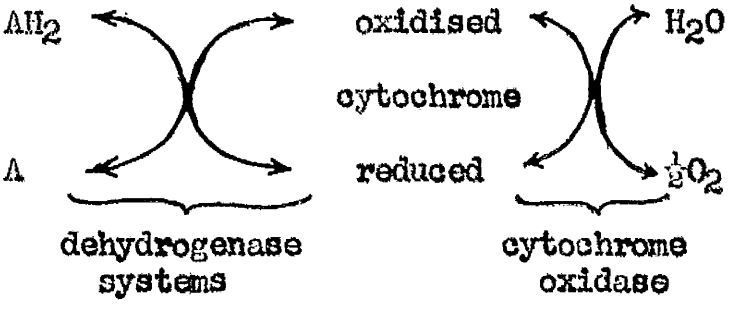
It will be seen from an examination of TABLE P that the protein contents of the various flour fractions are not related to the oxygen absorption values. The uptake during the second phase (5-20 min.) for Flour Series 9, is similar for each protein fraction and represents only 44% of the uptake of the parent flour. This finding suggests that the oxygen absorption process has been disturbed by air classification in a manner unrelated to the final

protein content of the flour.

It is suggested, therefore, that the differences between the uptake of high and low protein fractions may be related to both the disturbance of the components of an oxidising system, and to unequal distribution of lipid material between the two fractions.

Effect of pH on the Uptake of Dissolved Oxygen by Flour Suspensions

The experiments were originally designed to determine the effect of cyanide on the uptake of oxygen by flour. It is well known that many dehydrogenase systems depend on cytochromes for electron transfer to oxygen. The action of the cytochromes may be simply expressed by the following scheme (179) :-



The cytochromes are inhibited by cyanide, thus blocking the dehydrogenase systems. Wheat germ is known to contain succinic, glutamic, and hexose phosphate dehydrogenases (180), and the addition of cyanide to a flour suspension should indicate if these enzymes are involved in the uptake of oxygen by flour. The results of the experiments are reported in TABLE Q.

TABLE OE O

Effect of pH on the Uptake of Dissolved Oxygen by Flour Suspensions

Flour	Uptake 0-5 min. ($\mu l.O_2.$)	Uptake 5-20 min. ($\mu l.O_2.$)	Total Uptake ($\mu l.O_2.$)	pH
Series 3 Control	418	354	772	6.12
Series 3 + 1×10^{-4} M. KCN	425	305	730	-
Series 3 + 5×10^{-4} M. KCN	302	358	660	-
Series 3 + 7.5×10^{-4} M. KCN	293	328	621	-
Series 3 + 1×10^{-3} M. KCN	328	23	351	7.88
Series 4 Control	227	372	599	6.13
Series 4 + 1×10^{-4} M. KCN	399	325	724	6.38
Series 4 + 1×10^{-3} M. KCN	205	6	211	7.60
Series 4 + 1.25 ml. 2% KOH	180	5	185	7.16

The initial uptake (0-5 min.) of Flour Series 3 was not significantly affected by 1×10^{-4} M. KCN, but a similar concentration produced a stimulatory effect on the uptake of Flour Series 4. An increase of cyanide concentration to 1×10^{-3} M. inhibited the uptake of both flours, the effect being most marked during the second phase of uptake.

The addition of a concentrated solution of cytochrome 'c', and flour solubles equivalent to 3.25g. of flour, did not produce

an increase in the uptake of the flour suspensions containing 1×10^{-3} M. KCN.

In view of these results the pH of the suspension was checked to determine if the natural buffering capacity of the flour was being swamped by the addition of KCN. It was found that KCN increased the pH of the suspension as indicated in TABLE Q. Accordingly, the pH of the suspension was adjusted with potassium hydroxide to a similar value as found with 1×10^{-3} M. KCN. At pH 7.16, similar results were obtained by the addition of potassium hydroxide as by the addition of potassium cyanide. This finding indicates that the results are due to a change in pH rather than the inhibition of a cytochrome system. From the relative amounts of oxygen taken up during the period 0-5 min. and 5-20 min. it is found that the rapid initial phase is not as sensitive to pH changes as the final phase.

Smith and Andrews (11) have observed that the optimum pH for oxygen absorption by flour doughs is pH 6.7. Above pH 7.0 these workers found a sharp decrease in the amount of oxygen taken up by the dough. The findings reported above would indicate that an increase in the pH of the flour suspension from pH 6.1 to pH 7.2 produces a decrease in the oxygen absorption capacity of the flour.

GENERAL CONSIDERATIONS

GENERAL CONSIDERATIONS

The uptake of oxygen by wheat flour is the overall result of a number of oxidative processes which are inter-related in a complex pattern. The nature of the experiments has made it possible for certain general conclusions to be drawn as to the significance of lipid and protein constituents of flour in determining the magnitude and rate of oxygen absorption.

Flour takes up dissolved oxygen when wetted to the extent of about 710 µl./6g./20 min., of which about two-thirds has been absorbed by the flour after 5 min. The rapid initial phase appears to be concerned with both lipid and protein oxidation. The evidence supporting rapid lipid oxidation is based on the results obtained with defatted flours, and on the readdition of lipid to defatted flours. Defatted flours take up less oxygen than control flours and the decrease in uptake is noticeable during the initial phase. The restoration of the original flour lipids or the addition of linoleic acid to extracted flour increases the uptake, and in the case of linoleic acid it is only the initial phase of uptake which is affected. The amount of oxygen involved in lipid oxidation is difficult to assess, but it is probably in the region of 250 - 285 µl.O_2./6g. or about 35 - 40% of the total uptake. The rapid oxidation of flour lipids has been demonstrated during the mixing of flour doughs (137) and in

flour-water sponges (12). This oxidation has been related to the activity of lipoxidase (11,137) and to lipoxidase plus concurrent enzymic oxidation of all free fatty acids (12). The results reported in this thesis indicate that lipid oxidation also occurs in flour suspensions and were obtained by measurement of oxygen consumption, whereas previous results have been obtained by gas chromatographic analyses of lipid composition (12), or by the chemical estimation of the products of lipid oxidation (137).

The initial uptake of oxygen by flour is not completely accounted for by lipid oxidation. About 470 $\mu l.O_2./6g.$ are taken up during the period 0 - 5 min. after wetting, therefore about 200 $\mu l.O_2./6g.$ are taken up by a 'non-lipid' mechanism. The nature of the 'non-lipid' mechanism is unknown, but it is probably connected with the oxidation of the protein constituents of flour. The results reported in the literature indicate that sulphydryl oxidation could only account for a minute fraction of this uptake (119).

The rapid initial uptake of oxygen is followed by a slower phase (5 - 20 min.), during which about 240 $\mu l.O_2./6g.$ are absorbed. This phase is influenced by the prior treatment of the flour with oxidising agents and by the presence of lipids. The treatment of flour with improving or bleaching agents decreases the overall amount of oxygen taken up by the flour, the effect being most noticeable during the slower secondary phase. The effect of

both classes of agents is similar, which indicates that certain sites have been oxidised in a non-specific manner. In the case of improving agents the complete oxidation of sulphydryl groups could not account for the magnitude of the observed effect. The decrease in uptake on treatment is of the order of 150 $\mu l.O_2./6g.$ of which 115 $\mu l.O_2./6g.$ represents the decrease during the secondary phase. Lipids have a depressing effect on this phase as, on defatting, the amount of oxygen taken up during the period 5 - 20 min. is increased. This finding may be related to the observations of Narayanan and Mlynka (138) who have found that lipids exerted a protective influence against the improving effect of oxygen as measured by the rheological properties of dough.

The effect of nordihydroguaiaretic acid and sulphydryl blocking agents are difficult to interpret into a general theory. Nordihydroguaiaretic acid does not inhibit lipid oxidation in the flour suspensions studied, and its effect may be related to its ability to be oxidised. Sulphydryl blocking agents bring about an acceleration of oxygen absorption but do not greatly affect the amount of oxygen taken up by the flour. Thus, sulphydryl groups of flour have an influence on the oxygen uptake process which is not related to their ability to undergo aerobic oxidation. Glutathione undergoes oxidation in flour suspensions and it would appear probable that there are two types of sulphydryl groups in flour, those undergoing aerobic oxidation (65,119,133), and those which have a retarding influence on the rate of oxygen absorption.

Impact milling does not affect the oxygen absorption by flour, but after air classification the uptake of the high protein fraction is greater than that of the low protein fraction. The difference in uptake is found during the initial phase, and this may be related to the greater lipid content of this fraction (165,176). The oxygen uptake by both fractions is less than that of the parent flour and is unrelated to the gross protein contents.

From certain of the above considerations a general outline of the processes involved in the oxygen uptake by flour suspensions may be put forward (see p.159).

This scheme may be compared with the earlier findings of Smith and Andrews (11). These workers found that the uptake of oxygen by flour doughs was principally dependent on the oxidation of polyunsaturated fatty acids catalysed by lipoxidase. Morrison has since attributed the uptake to a more general lipid oxidation pattern (12). The uptake of oxygen by the protein components of flour has received less attention in the literature, although it is of greater significance in flour technology. Smith, van Buren and Andrews (133) noted that defatted flour doughs responded to mixing in oxygen and more recently Narayanan and Hlynka (136) have also demonstrated this effect. The oxygen response of defatted flours in the Rank and Hay batter process (2) was related by Hawthorn and Todd (3) to direct uptake of oxygen by flour proteins, although it was not possible to ascertain the amount of oxygen

General Scheme of Oxygen Uptake by Flour

Initial Uptake 0 - 5 min.	Secondary Uptake 5 - 20 min.	Overall Uptake 0 - 20 min.
250-285 µl.O_2. Lipid Oxidation → 470 µl.O_2. ← 200 µl.O_2. 'Non-Lipid' Oxidation	240 µl. O_2. Protein Oxidation	710 µl. O_2.
Not greatly affected by treatment	Decreased by treatment	Decreased by treatment
Decreased by defatting	Increased by defatting	Decreased by defatting

Notes

(1) This scheme is based on the mean values for oxygen uptake as reported for Flour Series 1 - 9 (TABLE A) and refers to the uptake of 6g. of flour.

(2) The values for lipid oxidation were obtained from a study of defatted flours (Flour Series 1 - 3) see p. 131 - 133.

involved in the improvement. The findings of Hawthorn and Todd (3) were criticised by Glass (129) and Learmonth (130) on the grounds that defatting was incomplete. It has since been shown that only the 'free' fatty acids are oxidised in flour doughs (11,12,137) and therefore the conclusions of Hawthorn and Todd are valid as the defatting procedure adopted by these workers would have removed 'free' lipid constituents. The results reported in this thesis support the conclusions of Hawthorn and Todd in that the uptake of oxygen should be regarded as the outcome of a general oxidation involving not only lipid but other flour constituents.

The relevance of these results to the technologist may now be considered. Recent trends in the baking industry are towards continuous automatic processing. The main features of this processing involve the development of dough during continuous mixing and the use of fast acting improving agents. These features eliminate the time consuming steps of fermentation and development as previously understood. To obtain maximum benefit of these innovations the chemistry of dough maturation needs to be understood. The literature reveals that the biochemical reactions which occur during maturation are still ill-defined.

The oxidation of certain flour components is known to bring about beneficial changes in the rheological properties of doughs. The nature of the improvement is believed to involve the sulphydryl groups of flour protein. This theory seems to have directed current

research into rather rigid channels and it is clear that sulphydryl oxidation is regarded as a 'magic formula' to explain improver action. This situation has probably arisen due to research into the structure of proteins in general, which has revealed the presence of disulphide bridges linking protein molecules. There can be little doubt that sulphydryl interchange and oxidation are involved in the improvement effect, and yet the oxidation of other flour constituents cannot be ignored.

The Rank and Hay 'batter' process (2) demonstrates the important effect of oxygen during mixing to bring about bleaching and improvement. This study of the uptake of oxygen by flour suspensions has indicated that the uptake process is complex and cannot be accounted for by sulphydryl or lipid oxidation alone. A more detailed study should reveal the presence of new centres for oxidation in flour and lead to a better understanding of the oxygen effect and a more knowledgeable application of oxidising agents in general.

C O N C L U S I O N S

CONCLUSIONS

The following conclusions refer to the uptake of dissolved oxygen by flour suspensions, studied by a polarographic technique.

1) Flour takes up dissolved oxygen from solution. The amount of oxygen taken up is of the order of 515 - 835 µl.O_2/6 g./20 min. depending on the flour studied.

2) Oxygen is rapidly taken up for about 5 min. after the flour has been wetted, thereafter the rate of uptake declines. After 20 min. the amount of oxygen being removed from solution is very small.

3) Treatment with normal amounts of improving or bleaching agents reduces the amount of oxygen taken up by the flour by about 20%. The reduction in uptake is more apparent during the slower secondary phase of uptake i.e. from 5 - 20 min. after wetting, than it is during the initial phase (0 - 5 min.).

4) Untreated flour, which has been extracted with petroleum ether, takes up slightly less oxygen than unextracted flour over the 20 min. experimental period. The amount of oxygen taken up during the initial phase is reduced. However, an increase is found in the amount taken up during the secondary phase. Methanol:chloroform (1:1, v./v.) extraction reduces the uptake of the flour to a greater extent than petroleum ether extraction.

5) As only a small reduction in uptake occurs on defatting untreated flours, the presence of a 'non-lipid' uptake mechanism is established.

6) Treated flour, extracted with petroleum ether, takes up less oxygen than treated unextracted flour. The decrease in uptake occurs during the initial phase, and the increase found during the secondary phase with untreated flours is diminished. Thus, 'free lipid' undergoes rapid oxidation when flour is wetted, and the amount of oxygen involved was found to be of the order of 250 - 285 $\mu l. O_2/6$ g. flour.

7) The addition of extracted lipids or linoleic acid to a methanol:chloroform extracted flour increases the oxygen uptake. Linoleic acid increased the uptake during the initial period after wetting, but did not affect the secondary phase significantly.

8) High levels of nordihydroguaiaretic acid do not inhibit the uptake of oxygen by the flour.

9) Sulphydryl blocking agents do not significantly affect the overall amount of oxygen taken up by flour, although these reagents do accelerate the rate of uptake. The addition of reduced glutathione to a suspension increased the uptake beyond a level expected if autoxidation had occurred.

10) Impact milling does not affect the uptake of oxygen by flour. The high protein fraction of air classified flour takes up more oxygen than the low protein fraction, but less than the parent flour. The uptakes are not related to the protein content of the flour.

11) The oxygen absorption process is sensitive to a pH change of from pH 6.1 to pH 7.2 .

SUMMARY

SUMMARY

The economic significance of wheat in human diet is largely attributable to the complex relationship which exists between its protein and lipid constituents. The technological properties of wheat flour are dependent on the susceptibility of these constituents to mild oxidation. The resulting reactions are of a complex nature but an evaluation of these reactions is a precursor to a more effective utilisation of this important crop as an article of diet.

The purpose of this study was to undertake an exploratory investigation into the uptake of dissolved oxygen by wheat flour suspensions. For this purpose flour suspensions were prepared by mixing a fixed quantity of flour and air equilibrated potassium chloride solution. An aliquot of the suspension was taken for study, and the uptake of dissolved oxygen was followed using a polarographic method. The electrodes were a rotating platinum microelectrode, which served as a cathode, and a saturated calomel electrode as anode. The potential applied to the cathode was regulated so that the current recorded on a sensitive ammeter was proportional to the concentration of oxygen in the suspension. Readings of oxygen concentration were taken at fixed intervals over the experimental period.

The results indicated that flour takes up oxygen rapidly when wetted. The amount taken up is influenced by commercial oxidative treatments and by the removal of flour lipids. The addition of extracted lipids and linoleic acid increased the uptake of defatted flour. A high level of an antioxidant (NDGA) did not inhibit the uptake mechanism. Sulphydryl blocking agents accelerated the uptake, but did not influence the overall amount of oxygen taken up by the flour. The addition of reduced glutathione increased the uptake of oxygen in the suspension. Impact milling did not affect the uptake, but differences were noted in the uptake of air classified high and low protein fractions. There was some evidence to suggest that the uptake process was pH sensitive. The results are discussed and related to the theories and findings of other workers.

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APPENDIX

APPENDIX

CALIBRATION OF POLAROGRAPH GALVANOMETER IN TERMS OF DISSOLVED OXYGEN CONCENTRATION

PART 1

Determination of Dissolved Oxygen

Dissolved oxygen in the electrolyte, 0.125 M.KCl, was determined by the Winkler method (1, 2-6).

Solutions required:-

(1) Manganous chloride ($MnCl_2.4H_2O$) 40g./100 ml. deionised water.

(2) Alkaline potassium iodide, 36g. NaOH +
10g. KI/100 ml. deionised water.

(3) Concentrated hydrochloric acid.

(4) Sodium thiosulphate 0.01N., freshly prepared and standardised.

(5) Starch solution - as indicator.

The Sample

0.125 M.KCl solution was prepared from a stock solution of 1.125 M.KCl. Duplicate solutions, prepared from 50 ml. stock 1.125 M.KCl + 400 ml. deionised water, were placed in 1 litre glass jars provided with rubber bungs carrying air inlets and outlets. The jars were placed in a thermostatically controlled water bath at 25° C., and the solution aerated for a period of 30 min. with a slow stream of air from an electrically operated pump.

The sample taken for analysis should completely fill the flask in which the titration is carried out. 250 ml. conical flasks with ground glass stoppers were found to be suitable for the determination. The volume of these flasks, when completely full and stoppered, was found by weighing with water at 25°C.

Results:-

Volume of Flask A = 284.7 cc.

Volume of Flask B = 281.65 cc.

Winkler Titration

A portion of the 450 ml. 0.125 M.KCl. was poured into a conical flask until it overflowed. 2 ml. manganous chloride solution and 2 ml. alkaline iodide solution were simultaneously added, by means of pipette to the bottom of the flask, 4 ml. of displaced sample being allowed to overflow. The flask was shaken thoroughly, and the precipitate allowed to settle out. 2 ml. of hydrochloric acid were added and the contents of the flask mixed thoroughly until the precipitate had completely dissolved. Titrations were carried out with a 100 ml. aliquot of this solution against 0.01N. thiosulphate, using starch as indicator.

Equations

$$MnCl_2 + 2NaOH \longrightarrow Mn(OH)_2 + 2NaCl$$

$$2Mn(OH)_2 + O \longrightarrow 2MnO.OH + H_2O$$

$$2MnO.OH + 6HCl \longrightarrow 2MnCl_2 + 4H_2O + Cl_2$$

$$2KI + Cl_2 \longrightarrow 2KCl + I_2$$

Results

$$O_2 \text{ in p.p.m.} = \frac{8 \times 1000 \times .01 \times T}{100}$$

$$= 0.8T$$

(Where T = Titre of 0.01N.thiosulphate)

Correcting for displacement:-

For Flask A:- $0.8T \equiv \frac{0.8}{1} \times \frac{284.7}{280.7} T \equiv 0.8114T$

For Flask B:- $0.8T \equiv \frac{0.8}{1} \times \frac{281.65}{277.65} T \equiv 0.8115T$

Titre for 100 ml. from Flask A = 10.00 ml. 0.01N.thiosulphate

Titre for 100 ml. from Flask B = 9.925 ml. 0.01N.thiosulphate

O_2 in p.p.m. (A) = 8.114 at 25° C.

O_2 in p.p.m. (B) = 8.054 at 25° C.

Mean of 8.08 p.p.m. dissolved oxygen (25°C.) or

6.18 ml./litre (25° C.).

PART 2

Calibration of the Polarograph

A series of four solutions of 0.125 M.KCl were prepared, and aerated for 30 min. at 25° C. in the water bath. Samples of 150 ml. were withdrawn and transferred to the polarograph cell. The rubber bung carrying the electrodes was inserted, and the assembly placed in a second water bath. The steady reading for the diffusion current of oxygen was recorded at 2.5 min. intervals over a 20 min. period using a galvanometer sensitivity of 1/30, and an applied voltage of -0.6 volts vs. S.C.E. The residual current was determined by the addition of sodium sulphite to the electrolyte.

Results

Mean reading for diffusion current = 52.6 scale divisions.

Mean reading for residual current = 0.0 scale divisions.

$\therefore$ 52.6 scale divisions $\equiv$ 6.18 ml. O_2./litre

or 1 scale division $\equiv \frac{6180}{52.6}$ µl.O_2./litre.

In the experiments with flour suspensions 150 ml. of suspension were taken,

$\therefore$ Absolute oxygen concentration for 150 ml.

$$= \frac{6180}{1} \times \frac{150}{1000} \text{ µl. } O_2.$$

$$= 927 \text{ µl. } O_2.$$

$$\therefore \text{ 1 scale division} \equiv \frac{6130}{1} \times \frac{150}{1000} \times \frac{1}{52.6} \ \mu l.\ O_2.$$

$$\equiv 17.62 \mu l.\ O_2. (\text{Log. } 1.2461)$$

Notes on Calibration Result

(1) A value of 8.7 p.p.m. for dissolved oxygen in 0.125 M.KCl at 20° C. has been recently reported by Bishop (7), who employed this solution as a standard to calibrate a similar polarographic system. This value corresponds to 6.12 ml. O_2./litre at 25° C. according to the tables of Tyler and Karchmer (8). These latter authors used a calibration solution whose oxygen content was 8.1 p.p.m. at 26° C.

(2) The oxygen diffusion current for 1.0M.KCl was recorded on the galvanometer in a similar manner to that described above. By means of the calibration factor, the value of the current was found to represent an oxygen concentration of 4.47 ml./litre at 25° C. The oxygen content of a similar solution has been reported as 4.26 ml./litre at 25° C. by Warshowsky and Schantz (9).

(3) Armstrong et al. (10) reported that the accuracy of the polarographic method for the determination of dissolved oxygen is within the range ± 3.4%, compared with that found using the Winkler method as a standard.

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